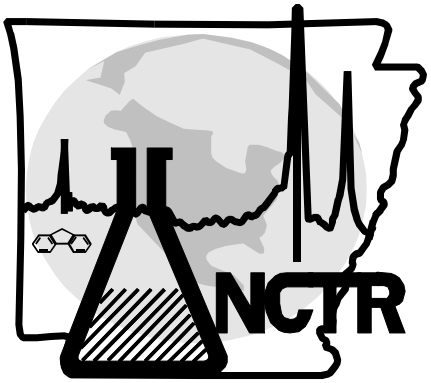
NCTR Research Accomplishments and Plans

FY 1996-1997



Leaders in Health Science Research for FDA

This document is compiled by the NCTR Planning Staff. To obtain additional information about the Center and/or additional copies of this document, you may contact the NCTR Planning Staff , 3900 NCTR Drive, HFT-321, Jefferson, AR 72079-9502; 501-543-7359 (phone); 501-543-7757 (fax); JANSON@FDANT.NCTR. FDA.GOV (Email); http://www.fda.gov/nctr/ (World Wide Web Location)

TABLE OF CONTENTS

PREFACE

The National Center for Toxicological Research (NCTR) is an FDA research facility located near Jefferson, a rural community in south central Arkansas approximately 30 miles from Little Rock. The mission of NCTR is to conduct peer-reviewed scientific research that supports and anticipates FDA's current and future regulatory needs. This involves fundamental and applied research specifically designed to define biological mechanisms of action underlying the toxicity of products regulated by FDA. This research is aimed at understanding critical biological events in the expression of toxicity and at developing methods to improve assessment of human exposure, susceptibility and risk.

NCTR conducts integrated research with other FDA centers and leverages FDA resources through cooperative and/or collaborative agreements with other agencies, academia and industry. These interactions enhance opportunities to provide more effective risk measures for FDA-regulated products and support FDA enforcement through methods development.

NCTR research is focused within three strategic research goals:

> The development of knowledge bases (KNLG) or the accumulation of data that have predictive values extending beyond the individual data elements and which foster the identification of data gaps and new research areas leading to the development of predictive systems in support of regulatory decisions.
>
> The development of new strategies for the prediction of toxicity (PRED) based on mechanism-based assays that contribute to a profile of information that supports a regulatory decision.
>
> The conduct of method- (METH), agent- (AGNT), or concept-driven (CNPT) research or the modification and development of better analytical and toxicological test methods, and the provision of data on specific agents of interest to FDA to facilitate current and anticipated regulatory needs.

NCTR research is conducted within the auspices of eight research divisions whose goals, ongoing research accomplishments and FY97 plans are summarized herein. All NCTR research is directed toward the resolution of scientific and regulatory issues that provide the basis for regulatory decisions.

An NCTR extramural Science Advisory Board, its subcommittees and liaison members from each of the other FDA centers/ORA actively provide guidance on the relevance and quality of these research efforts.

B.A. Schwetz, D.V.M., Ph.D.
Director, NCTR

SCIENCE ADVISORY BOARD

SCIENCE ADVISORY BOARD
TO THE
NATIONAL CENTER FOR TOXICOLOGICAL RESEARCH

Function

One of the keys to maintaining a high quality research organization is the utilization of an outside body of experts to periodically review the quality as well as the direction of the research. The Board assists the NCTR Director and the FDA Commissioner by providing recommendations in this regard.

FY 96 Accomplishments

As part of its ongoing review of scientific programs at the NCTR, members of the Science Advisory Board (SAB) held a full board meeting January 29-30, 1996. The Board took a look back at the previous eight site visits that they had completed in an attempt to put into perspective the "whole" of the NCTR research agenda that was represented by the eight parts. The Center/ORA liaisons participated in this discussion by trying to outline for the Board their respective Centers' research priority agendas and how they were interconnected with work of the NCTR in order to provide the Board with some perspective as to the relevancy of the Centers' programs. As part of this discussion the Board received a description of the support services activities that are accomplished by on-site contract employees and represent more than 50% of the staff at the Center.

The SAB concluded from their assessment that the site visit process had been a success and it will continue to review the NCTR science and its relevancy on a periodic basis. A site visit review approximately every three to five years was believed to be sufficient.

SCIENCE ADVISORY BOARD TO THE NCTR
Membership Roster

NAME/TITLE	AFFILIATION	TERM ENDS	EXPERTISE
Dr. Marion W. Anders* Professor, Chairman, Dept. of Pharmacology	University of Rochester Rochester, NY	6/30/98	Veterinary Medicine, Biochem./Pharm.
Dr. Robert E. Anderson Professor, Emeritus, West Virginia University	WV School of Environmental Education, Inc.	6/30/00	Food Technology
Dr. William R. Bruce Professor, Departments of Medical Biophysics and Nutritional Science	University of Toronto Don Mills, Ontario	6/30/99	Medicine, Biophysics
Dr. Harold Davis Director of Toxicology	AMGEN Thousand Oaks, CA	6/30/98	Pathology, Veterinary Medicine
Dr. Tómas R. Guilarte Professor, School of Hygiene and Public Health	Johns Hopkins University Baltimore, MD	6/30/99	Medical Physics, Zoology
Dr. Joseph V. Rodricks Senior Vice President	ENVIRON International Corporation Arlington, Virginia	6/30/99	Toxicology/Risk Assessment
Dr. Marcy E. Rosenkrantz Associate Director	Cornell Theory Center, Cornell University	6/30/00	Computational Chemistry
Dr. Charles L. Wilkins Professor of Chemistry and Assoc. Dean Physical and Mathematical Science	University of California, Riverside	6/30/00	Chemistry
Dr. Lily Y. Young Professor of Microbiology AgBiotech & Department of Environmental Sciences	Rutgers University Cook College New Brunswick, NJ	6/30/98	Microbiology
Mr. Ronald F. Coene Executive Secretary Deputy Director, Washington Operations, NCTR	FDA/NCTR Rockville, MD	Ongoing	Research Administration

*Committee Chair

RESEARCH ACCOMPLISHMENTS AND PLANS

BIOCHEMICAL TOXICOLOGY

BIOCHEMICAL TOXICOLOGY

Director: Frederick A. Beland, Ph.D. **Telephone: 501-543-7205**

Introduction

This year one out of every five deaths within the United States will be due to cancer. Since it has been estimated that 60-90% of these cancers result from exposure to environmental or exogenous agents, a significant proportion of this disease should be preventable through controlling exposure to these factors. Cigarette smoking, which accounts for nearly 30% of all cancer deaths, is clearly the major identifiable cause of cancer in our society. The agents responsible for the remainder are more varied and are believed to be due primarily to trace substances found in food, water, air, medicines, and cosmetics. A number of substances carcinogenic to animals, including mycotoxins, nitrosamines, urethanes, heterocyclic aromatic amines, and hydrazines, have been identified in foods and beverages. Likewise, in recent years, certain widely used drugs, such as phenacetin, methapyrilene, methylphenidate, 3'-azidothymidine (AZT), and tamoxifen have been demonstrated to be carcinogenic in animals. Considering the amounts of food, beverages, and drugs that are consumed, along with the carcinogenic potency of substances contained within these groups, it appears that these three sources are potential, major contributors to the incidence of human cancers.

FY 97 Goals

In the area of biochemical toxicology, a major focus of research is to assist other FDA centers in their regulatory mandate by: 1) learning about the process of cancer; and 2) leading the FDA in the introduction of new techniques to assess carcinogenic risk.

The identification of carcinogens has depended classically upon two approaches, epidemiological studies and chronic animal bioassays, each of which has its own strengths and weaknesses. Thus, while epidemiologic techniques are clearly capable of identifying human carcinogens, these determinations are typically made after the cancer has arisen,

which is hardly an ideal situation. And, while animal bioassays are useful for indicating the potential carcinogenicity of chemicals, there are a number of uncertainties concerning the extrapolation of animal data to humans. For example, since only relatively small numbers of animals can be used in bioassays, suspected carcinogens are administered at doses that typically exceed human exposures. In order to assure that the responses detected in the bioassays are germane to humans, a clear understanding of the mechanisms or process of cancer induction in animal models is necessary. This is a major focus of the Division of Biochemical Toxicology.

A central tenet of cancer research is that tumors arise from cells that have undergone a permanent heritable change in their DNA. Although a number of mechanisms can be envisaged to explain the origin of these heritable changes, clearly a dominant theme in biochemical toxicology research is that, in most instances, they arise from the interaction between chemical carcinogens and DNA to form DNA adducts. As such, the elucidation of the structures of these adducts can provide essential information concerning the metabolic activation pathways of suspected carcinogens. Furthermore, by determining the identity, quantity, and persistence of DNA adducts, insight can be obtained on the effects of the adducts on DNA structure, transcription, synthesis, and repair. Investigations of the specific types of mutations induced by particular DNA adducts can provide a direct test for the role of DNA adducts in carcinogenesis. Finally, DNA adducts can be used as dosimeters to estimate the relative risk for tumor induction.

While recognizing the importance of exogenous DNA adducts, not all carcinogens induce cancer through their direct interaction with DNA. Additional mechanisms include, for example, perturbations in cell cycle kinetics, the induction or suppression of cell death, the initiation of lipid peroxidation with concomitant formation of endogenous DNA damage, and perturbations in DNA methylation which could lead to alterations in gene expression. Clearly, an accurate understanding of carcinogenic risk requires assessing the potential contributions of these factors.

FY 96 Accomplishments

While acknowledging the limitations of animal bioassays, these studies currently serve as the benchmark by which toxicological assessments are made by federal agencies, including the FDA. The NCTR has animal facilities that are rivaled by few, if any, research institutions. As such, the Center has the capability to conduct subchronic and chronic toxicological assessments in a rigorous manner to address the Agency's needs. In addition to providing basic information on toxicological endpoints, such

as cancer, these studies serve as the basis for mechanistic studies to ascertain if the response detected in the experimental model is pertinent to humans.

In response to requests made to the National Toxicology Program (NTP) by the Center for Drug Evaluation and Research (CDER) and the Center for Food Safety and Applied Nutrition (CFSAN), chronic bioassays were conducted during FY96 on the pediatric sedative, chloral hydrate, and the mycotoxin, fumonisin B_1 (FB_1). The results of these ongoing bioassays will be used by the centers to establish the risks associated with exposure to these compounds, which will form the basis for regulatory decisions. Likewise, bioassays on nitropolycyclic aromatic hydrocarbons, air particulate samples, coal tar, and benzo[*a*]pyrene have been conducted to assist other agencies in their regulatory mandate. As an example, a risk assessment was performed using the tumorigenicity data acquired with benzo[*a*]pyrene and coal tar. In addition, at the request of the Center for Veterinary Medicine (CVM), subchronic bioassays were initiated on malachite green, a therapeutic agent used in aquaculture.

Traditional chronic carcinogenicity bioassays are both very expensive and lengthy; thus, the development of alternative methods of assessing carcinogenic potential would be of great value. One approach that is currently being investigated is the neonatal mouse tumorigenicity assay. The advantages of this method are that only limited amounts of test material are required, a direct assessment is obtained as to whether or not the agent acts through a genotoxic mechanism, and less time is required to elicit a carcinogenic response. Currently, in collaboration with investigators at CDER, this alternative bioassay is being applied to selected benzodiazipenes and antihistamines. In addition to the neonatal mouse assay, a cell transformation laboratory has been organized around two general themes: first, that cell transformation systems can be used for identification or evaluation of hazards of interest to the FDA; and second, these systems can provide relevant mechanistic information concerning the multistage nature of carcinogenesis *in vivo*. During the current year, efforts in this area centered around the effects of extremely low frequency magnetic fields on nuclear matrix proteins, a topic of interest to the Center for Devices and Radiological Health (CDRH).

As noted above, the covalent interaction of carcinogens with DNA is a common critical event in carcinogenesis. If the structure of the adduct is known, the appropriate immunogen can be synthesized and antisera can be raised. Within the biochemical toxicology area, an ongoing goal is to exploit both the immunogenicity and the antigenicity of these adducts to develop and apply immunochemical methods to address problems of regulatory concern including exposure, risk of toxicity, product screening, and mechanisms of toxicity. During the current year, this technology was applied to fumonisin B_1 and sulfonamide drug residues.

Recently, much public attention has been given to the controversy over the safety of silicone breast implants. An even larger issue is a general dearth in knowledge concerning the safety of biocompatible materials. A goal of the biochemical toxicology group is to establish a strong research effort in this relatively neglected area. This will increase knowledge of the

mechanisms of long-term toxicity of implanted materials and thus increase the scientific validity of regulatory decisions pertaining to the safety of materials intended for prolonged residence in the body. Efforts during the current year have focused on evaluating if serum albumin adducts can serve as biomarkers for exposure to toluenediamines, carcinogenic aromatic amines that have been observed in women with polyurethane-covered breast implants.

In order to help interpret the carcinogenicity bioassays being conducted with fumonisin B_1, a number of additional studies were conducted, including investigating the mechanism of action of fumonisin B_1, understanding the pharmacokinetics of fumonisin B_1 in rodents and nonhuman primates, identifying the genotoxicants in *Fusarium*, and monitoring experimental animals and exposed human populations for *Fusarium*-derived DNA adducts. Similar mechanistic studies have been conducted with chloral hydrate, malachite green, polycyclic aromatic hydrocarbons, nitropolycyclic aromatic hydrocarbons, and aromatic amines.

Although most tumors arise from the covalent interaction of chemical carcinogens with DNA, the correlation between the concentration of DNA adducts and the resultant tumorigenic response is by no means certain. This relationship has been elucidated using tumor models in which potential human carcinogens are administered over wide dose ranges. The advantage of this approach is that dose-response relationships can be established at doses where a statistically significant increase in tumors cannot be detected by conventional bioassays. DNA adducts exert their deleterious effects as a consequence of DNA replication and cell proliferation; however, experimental data indicate that not all DNA adducts have the same carcinogenic potential. The reasons for these differences are not completely understood and have been investigated through the application of molecular biological techniques to determine the specific mutations that result from DNA adducts as well as the distribution of adducts with specific DNA sequences. In addition, sophisticated spectroscopic techniques have been used to establish the conformational effects of adducts upon oligonucleotide sequences associated with oncogenes, and these effects are being related to the specific mutations that result. This information will allow greater confidence in risk estimates based upon DNA adduct determinations.

FY 97 Plans

Agent-Method- Driven Research

During 1997, chronic bioassays on the NTP-nominated chemicals fumonisin B_1 and chloral hydrate will be finished. In addition, a chronic bioassay with malachite green will begin. Likewise, in response to a nomination by CFSAN, chronic studies will start to examine the ability of ethanol to potentiate or attenuate the carcinogenicity of

urethane, an agent found in alcoholic beverages. A major new initiative for the division will be in the area of endocrine disruptors, and during the year subchronic studies will be conducted with methoxychlor and genistein.

Mechanistic studies will also continue on fumonisin B_1 and chloral hydrate, with the emphasis on helping interpret the results of the bioassays and determining their applicability to humans. With regard to chloral hydrate, emphasis will be focused on the induction of endogenous DNA damage, on attempting to understand the differences in species susceptibility, and on determining the specific metabolites responsible for the tumorigenicity of this pediatric sedative. Mechanistic experiments on fumonisin B_1 will be centered on the role of apoptosis in the tumorigenic response and upon the formation of DNA adducts by other mycotoxins produced by *Fusarium*. In addition, by taking advantage of antibodies generated against specific regions of the fumonisin B_1 molecule, studies will be initiated to determine the intracellular localization of the mycotoxin and to purify ceramide synthetase, an enzyme thought to be involved in toxicities of fumonisin B_1. Studies with malachite green will include investigations of the dye's metabolic pathways, with particular emphasis on thyroid homeostasis. Mechanistic investigations on the interactions of ethanol and urethane will focus on alterations in DNA adduct levels under conditions that mimic the bioassay, and on the ability of ethanol to alter cell cycle kinetics.

Concept-Driven Research

In the area of biomaterials, studies will continue to determine if sexual dimorphisms in the immune system could have significant effects on the inflammatory response to implanted biomaterials. The results from this study could be significant for the testing of biomaterials and for elucidating the mechanisms of foreign-body tumorigenesis. As part of the investigations into foreign-body carcinogenesis, experiments will be conducted to elucidate the roles of oxidative DNA damage and cytokine expression that result from the inflammatory response on the initiation and progression of tumorigenesis. Finally, the degree of fibrotic response is important to the long-term success of an implanted medical device and appears to be important in the development of tumors near a foreign body. Since it is unclear what controls the extent of this response, this issue will be addressed by comparing species differences with regard to the nature of the response to a given material.

New Strategies for the Prediction of Toxicity

Investigations will also continue to determine the effect of carcinogen structure upon the conformation of DNA and upon the factors that govern the repair of DNA adducts within specific nucleotide sequences. Efforts will be undertaken to understand the age-specific changes in gene expression, with emphasis on susceptibility to carcinogens. And finally, immunochemical methodology will be combined with mass spectral technology to improve the

identification of low levels of chemicals, their metabolites, and reaction products with DNA and other macromolecules.

Significance to the FDA

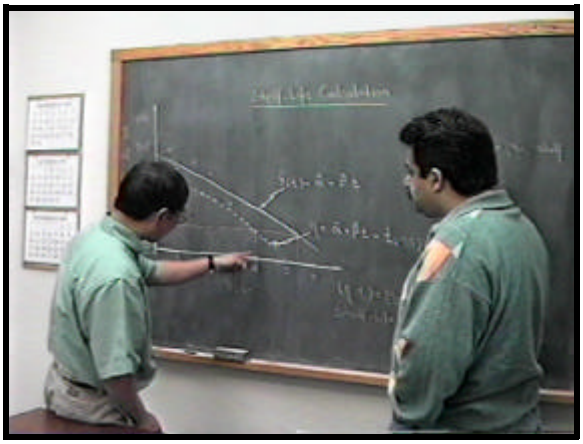
The FDA is entrusted with the responsibility of insuring the safety of foods, drugs, biologics, medical devices, and cosmetics. Animal bioassays can indicate whether or not chemicals associated with these products are carcinogenic; however, extrapolations from experimental animals to humans are difficult due to differences in dose, as well as length, frequency, and route of exposure. By characterizing the activation pathways of carcinogens associated with foods, drugs, and other products, the projects in this area are attempting to establish if the metabolic pathways important in animal models are relevant to humans. The experiments will also suggest if certain populations are at increased risk and should therefore be the focus of regulatory decisions. A direct estimate of the biologically effective dose of a carcinogen can be obtained by conducting DNA adduct measurements in humans, which should be invaluable for determining if animal carcinogens are indeed posing a risk to exposed individuals. Furthermore, by using DNA adducts as biomarkers, there will be greater confidence in describing the shape of the low end of the dose-response curves for carcinogens. This in turn should provide increased confidence in extrapolating from high-dose animal bioassays to low-dose human exposures. By characterizing the effects of DNA adducts, a direct measurement of the biological response to exposure can be obtained. Finally, since not all carcinogens induce cancer through their direct interaction with DNA, an accurate understanding of carcinogenic risks associated with particular chemicals requires assessing the potential contribution of other factors, such as perturbations in cell cycle kinetics, the initiation of lipid peroxidation, or the induction of cell death.

BIOMETRY AND RISK ASSESSMENT

BIOMETRY AND RISK ASSESSMENT

Director: Ralph L. Kodell, Ph.D. **Telephone: 501-543-7008**

Introduction



The regulation of toxic substances in foods, drugs, cosmetics, animal drugs, and medical devices requires quantitative risk/benefit analyses. Quantitative risk assessment is a data-based process for deriving numerical estimates of health risk (proportion of diseased individuals) as a function of the conditions of exposure to toxic substances. The daily dose rate, the age at exposure, and the duration of exposure are all factors that influence risk. Assumptions often are required in order to extrapolate results from the high doses that are utilized to elicit effects in animals, to the low doses experienced by humans, and to extrapolate across different routes and durations of exposure. Consequently, the uncertainty in cancer risk estimates can be as much as two orders of magnitude. Research is being conducted to reduce the uncertainties inherent in these assumptions in order to improve quantitative estimates of risk.

FY 97 Goals

1. Develop knowledge bases for constructing predictive measures of health risks through the use of multiple regression, discriminant analysis, and pattern recognition.

2. Develop new statistical strategies for prediction of the risk of cancer, developmental toxicity, and neurotoxicity.

3. Develop biologically-based dose-response models for growth of the developing limb and for mechanisms of cancer

4. Conduct method- and concept-driven statistical research on dose-response modelling, intermittent dosing, exposure to complex mixtures, interspecies extrapolation, and hazard identification, in order to improve the assessment of health risk.

5. Conduct agent-driven research on physiologically-based pharmacokinetic models of pregnancy.

6. Assist other FDA centers in obtaining quantitative risk estimates for the regulation of specific products and in investigating generic risk assessment issues.

7. Assist NCTR scientists in the statistical design for research projects and the statistical analysis of research data to evaluate the toxicity of regulated products.

8. Participate in interagency risk assessment activities to maintain knowledge of the state-of-the-art and to contribute to improving and unifying risk assessment procedures across agencies.

FY 96 Accomplishments

Scientists in the Biometry Branch either published or had accepted for publication, 27 first-authored research papers, and co-authored an additional 13 papers. Major research accomplishments under each of NCTR's strategic research goals were as follows:

Development of Knowledge Bases

A procedure was developed for detecting and alleviating the problem of collinearity under a proportional hazards regression model (E06974). The proportional hazards model is widely used to evaluate epidemiological data submitted to the FDA in support of petitions for new products.

A protocol was drafted (X70048) for conducting a pilot study on developing a knowledge base for predicting the outcome of the two-year cancer bioassay using results of short-term tests and structure activity relationships (SAR). The proposed approach is to exploit the structure of the Moolgavkar-Knudson-Venzon (MKV) model of carcinogenesis to identify predictor variables to be used in a logistic regression analysis. The ultimate intent is to provide a means to accelerate the approval process of human and animal drugs, food additives, and medical devices.

New Strategies for the Prediction of Toxicity

A statistical procedure was developed for calculating an upper bound on the sum of the risks of the components in a mixture of carcinogens, and a strategy for optimum risk reduction was

proposed (E06910). The procedure eliminates the undue conservatism inherent in the common approach of simply summing the upper bounds on risk for individual components.

An interim unified approach to safety assessment for both carcinogenic and noncarcinogenic effects was drafted (S00174). The approach is directed toward restricting the use of mathematical models to the range of observed data, and using a consistent method of extrapolating to acceptable levels of exposure, which de-emphasizes numerical estimates of risk.

Computerized images were captured from both serial sections of mouse fetuses and laser scanning confocal microscopy optical sections of mouse embryos (E06953). Routine collection of data is near. The short-term goal is to obtain embryonic limb development data for use in growth models

Method-Driven Research

Nonparametric tests for comparing tumor incidence rates in animal bioassays were developed for the multiple-sacrifice and single-sacrifice cases (E06870). A Monte Carlo simulation study showed that these tests have excellent statistical properties. Unlike the standard International Agency for Research on Cancer (IARC) methods, these tests do not require cause-of-death information. Hence, they are more broadly applicable, and more cost effective.

A theoretical framework and a computational procedure were developed for a physiologically based, pharmacokinetic (PBPK) model of pregnancy (E06638), which will incorporate data from four species.

Risk assessment methods for quantitative response data were compared to methods for quantal data and were extended to the case of mixtures of distributions of quantitative responses (S00116).

A linear mixed-effects model was developed for the analysis of data from drug stability studies (E06909). The model can be used to establish the shelf life of drugs from different batches and packagings thus, providing a more efficient method for utilizing analytical chemistry data on drug stability.

Concept-Driven Research

A new biologically based mathematical model for cancer was developed and refined (E06908). Unlike previous models, this model depicts the specific mutational events that take place at the nucleotide level. The model represents a significant advance in the mathematical representation of the cancer process.

Other important accomplishments of the Biometry Staff during FY96

During FY96, the Biometry staff collaborated with CDER on research to assess the statistical implications of conducting bioassays of shortened duration for drugs; to analyze chemistry data from drug stability studies, to evaluate the equivalence of drug dissolution profiles and to develop a points-to-consider document on biostatiscal methodology for carcinogenicity studies. A simulation study on reducing conservation in risk estimation for mixture of carcinogens was continued with CFSAN.

The Biometry staff consulted with other NCTR researchers on the design of studies in support of the NCTR strategic goal to conduct agent-driven research, specifically studies on fumonisin B_1, urethane, and antihistamines. Additionally, Biometry scientists consulted on the appropriate statistical techniques for analyses of data generated in studies involving caloric restriction and tumorgenicity, and on studies using flow cytometric data.

Biometry staff members, through invited presentations at both national and international meetings, workshops, and universities, have broadened the impact of NCTR research efforts to improve risk assessment for cancer and non-cancer endpoints. Biometry staff members have distinguished themselves as consultants and/or associate editors on peer reviewed statistics journals (Communications in Statistics - Theory and Methods, Journal of Agricultural, Biological and Environmental Statistics) during FY96.

FY 97 Plans

All ongoing projects which have not been completed will continue into FY97. In addition, some current projects will be expanded and several new projects will be initiated, as follows:

<u>Development of Knowledge Bases</u>

The draft protocol for X70048 will be finalized and implemented. The resulting pilot study will determine the feasibility of developing a knowledge base for predicting the outcome of the two-year cancer bioassay using results of short-term tests and SAR.

<u>New Strategies for the Prediction of Toxicity</u>

The Monte Carlo simulation study for the collaborative project with CDER (E06902) will be completed. Results will be evaluated with respect to the statistical implications of shortened bioassays for assessing the toxicity of drugs.

A joint project with CFSAN will be undertaken to investigate the feasibility of expanding the Threshold of Regulation for indirect food additives to include direct flavor additives.

Method-Driven Research

A protocol will be developed under X70045, to conduct a Monte Carlo simulation study to assess various tests for dose-related trend for exchangeable, binary data, such as litter data from developmental toxicity studies.

The new experimental protocol (E06984) on the statistical analysis and characterization of the joint action of toxicants will be implemented.

A protocol will be developed under X70046, to investigate statistical methods for adjusting p-values with respect to the testing of multiple endpoints.

A new Mathematical Statistician will be hired to provide expertise in statistical epidemiology. In addition to providing expert consultation on problems in epidemiology, that person will begin to develop a research program directed toward the development of improved statistical methods for evaluating epidemiology data.

A joint project with CFSAN will be undertaken to develop a quantitative model for the relationship between food consumption and body weight and to assess the effects of food restriction on pharmacokinetic parameters.

Concept-Driven Research

Work under E06908 will be expanded to include an investigation of molecular dosimetry within the structure of a model that specifically depicts mutational events at the nucleotide level.

Additional plans for consultation and collaboration in FY97 include:

Consultation on NCTR Experiments

In addition to ongoing consultations, a major effort will begin under E00501-E00509 to conduct a series of statistical analyses to evaluate body-weight, survival, and tumorigenicity data from the series of studies on Caloric Restriction in rats and mice.

FDA Collaborations

All ongoing FDA collaborations will continue throughout FY97, and opportunities to engage in additional collaborations or consultations will be explored. In particular, an attempt will be made to become more active in the FDA Statistical Association. In addition, division scientists will make an effort to establish liaisons with statisticians in additional FDA centers (e.g., CDRH).

Significance to the FDA

The NCTR has been designated by the FDA as the focal point for research in the area of Health Risk Assessment, including investigating the critical assumptions that underlie such assessment. Human health risk estimates impact on the regulation of exposure to toxic substances which affect the health and economy of the U.S. population. The Division of Biometry and Risk Assessment has the key role of identifying uncertainties in the risk assessment process, and developing risk estimation techniques to reduce these uncertainties and to improve the regulation of natural or synthetic toxic substances occurring in foods, drugs, cosmetics, and medical devices. Continued significance to the FDA is fostered through interactions with individuals and committees at other FDA Centers that are involved in evaluations of risk for the regulation of specific products.

CALORIC RESTRICTION

CALORIC RESTRICTION

Director: Ronald W. Hart, Ph. D. **Telephone: 501-543-7116**

Introduction

In the accomplishment of the FY 96 goals, it became clear that caloric intake significantly alters the efficacy of a number of key biological systems responsible for the animal's capacity to maintain homeostasis and deal with both endogenous and exogenous stress. These findings, taken together with previous ones, negate the assumption previously used in toxicology that, in the absence of data to the contrary, the toxicological effect of an agent, process or device was independent of diet. Therefore, it is now important that the NCTR program address two primary issues: first, the development of practical methods for implementation of these findings relative to product testing and evaluation; and second, the applicability of our previous findings to assessments and estimations of health risks in humans.

FY 97 Goals

Consistent with the above, and as an extension of our FY96 accomplishments, in FY97 the program will focus its goals into two major categories. The first will relate to applicability of previous findings to practical methods of implementation of these results in product testing. The second catagory relates directly to whether or not our previous findings are or are not consistent with similar changes induced in humans by similar levels of caloric restriction and nutritional supplementation. In order to achieve the streamlining of research activities, the program has either closed out or is in the process of writing final reports on all projects peripheral to these goals.

Category 1 Goals:

- Determine the relationship between body weight and tumor occurrence in animal model systems based on historical databases.

- Complete anaylsis of existing governmental animal bioassay databases from the NTP, FDA, and other sources in order to determine the impact of body weight differences on a number of physiological, biochemical, molecular, and pathological endpoints used in assessing the safety and toxicity of compounds, processes, and devices. As part of this goal, specific recommendations will be made as to practical methods for implementation of these findings in the routine analysis and conduct of animal bioassays.

- Determine the ideal level of caloric intake and develop methods of implementation for animal studies.

  - Evaluation of practical methods of implementation referred to above are critical for establishing external credibility of the suggested procedures. This process entails the conduct of a long-term animal bioassay under a set of conditions selected in order to compensate for differences in body weight between control and experimental animal groups. Such studies will permit better assessment of the impact of having failed to consider such differences on previous studies, provide a practical demonstration of how such body weight differences might be addressed, and directly demonstrate the efficacy of these procedures.

  - Assessment of this procedure will be, in part, dependent upon the ability to establish the most practical level of food intake needed to achieve a similar body weight in experimental and controlled animals with a minimal impact upon what is considered to be normal levels of physiological, biochemical, metabolic, and molecular parameters. In order to achieve this, a dose-response relationship will have to be established between the level of dietary intake and its impact upon these endpoints. This will require direct measurement of a selected number of these endpoints as a function of dietary intake in animal strains normally used in the two-year chronic bioassay. Additionally, survival characteristics and pathology profiles will be determined for Sprague Dawley rats using the newly developed AIN-93 purified diet. If successful, this diet will be used by CFSAN in clinical bioassays to assess the safety and efficacy of food additives and food substitutes.

Category 2 Goals:

Ultimately, the true test of experimental biological science is its applicability and application to human health. A unique opportunity has presented itself in that a human model system virtually identical to the animal experimental system used by the PCR group to elucidate the impact of dietary intake has become available to the group. This human model will be used to validate various physiological, biochemical, metabolic and molecular endpoints in humans. Additionally, this model has become available at a time when the PCR participants have established and evaluated the impact of dietary intake as a function of strain, sex, time of

day, and dose of caloric intake; thereby, providing an extensive database and availability of techniques applicable to human studies.
Since our PCR program approach is historically, by design, both multi-disciplinary and interdisciplinary, it cuts across a number of administrative lines of authority. Though its management activities are centered within a PCR core group that is composed of only seven FTE's, it involves personnel from a number of divisions across NCTR, and maintains active collaborations with over a dozen investigators across the country.

FY 96 Accomplishments

Category 1: Application studies.

- Analysis, review, evaluation, and interpretation of existing governmental animal bioassay databases.

    - During FY 96 significant progress has been made in the direct analysis of the raw data created during the conduct of a number of two-year animal chronic bioassays. The insights provided by these evaluations and a more rigid examination of the analytical procedures used in order to establish a relationship between body weight/organ weight and the probability of spontaneous tumor occurrence in various organs, sexes, strains, and species has been significant. While time consuming, this analysis provided a more solid base upon which adjustments can be made in relating body weight to tumor occurrence. These studies have further supported the hypothesis that caloric intake is directly related to body weight by its impact on a number of key biological processes including, cell proliferation and apoptosis. Further, it is now more apparent than ever that reduced caloric intake slows the time to occurrence of cancer and elevates the capacity of the system to either repair the resultant damage or eliminate the defective cell. It is important to note that a number of papers published during FY 96 further established a relationship between body weight changes in humans and the frequency and time to occurrence of similar pathologies of those observed in rodents to those observed in humans.

- Progress on determining the ideal level of caloric intake and its implementation for bioassay studies.

    - During FY 96, despite a significant delay in startup resulting from limited animal room access, significant progress was also made on the effect of diet and different levels of caloric restriction on physiological, metabolic, biochemical, immunological, molecular, and body composition variables in rats. The animals have been loaded and initial interim animal sacrifices made.

- During FY 96, the design and logistics for a study in the chronic bioassay of chloral hydrate in male $B_6C_3F_1$ mice using idealized body weight curves that are normalized by modulation of caloric intake was accomplished. The study has been implemented and excellent progress is being made. At the present time, it would appear that the procedures outlined in the proposal will be fully met.

Category 2: Relevance to humans.

- During the latter part of FY 96 a human clinical trial protocol was designed, developed, reviewed, and approved to study the impact of changes in caloric intake on a number of physiological, metabolic, biochemical, molecular, cellular, and pathological endpoints. These studies were designed in conjunction with our collaborators at the University of Tennessee School of Medicine in Memphis and Baptist Memorial Hospital, in Memphis. This latter group will provide material for analysis at NCTR. The hypothesis is that reduced caloric intake will result in a similar series of physiological, metabolic, biochemical, and molecular changes in humans as those observed in rodents.

FY 97 Plans

Category 1: Application studies.

- It is anticipated that during FY97, the points-to-consider documents would summarize the findings relative to the relationship of body weight and tumor occurrence with specific recommendations of how best to adjust for these factors. The documents will be once again reviewed and published by the agency-wide committee dealing with these matters.

Plans for FY 97 include: determining the chronic toxicity and potential carcinogenicity of chloral hydrate, administered by aqueous gavage, to male $B_6C_3F_1$ mice; and, continuing to establish the feasibility of utilizing dietary control (*i.e.,* the manipulation of caloric intake) to control body weight gain so that all mice in each experimental group of the bioassay conform to an idealized weight curve.

Plans for FY 97 include: determining how various levels and durations of caloric restriction (CR) affect physiological function, enzymes related to intermediary and drug metabolism, hormonal regulation, blood chemistry, gene expression, regulatory proteins (heat shock), immunological function, and pathological profiles to develop and redefine biomarkers of toxicity under CR conditions. In addition, it is planned that we will develop: a) a non-invasive method of determining body composition in rats; b) the impact of CR on qualitative and

quantitative changes in lipid storage and metabolism, as well as the distribution of fat deposition; c) develop experimental methods for utilizing CR in the chronic bioassay; and, d) compare the survival and pathology profiles between the AIN-93M purified diet and the NIH-31 natural formula diet.

Category 2: Relevance to humans.

The primary objective for FY 97 will be to determine whether rodents and humans behave biologically in the same manner when calorically deprived but nutritionally supplemented. More specifically, the study will determine whether the set of biological markers developed in rodents as predictors of disease can also be related to overall human health and well being. These biological markers include but are not limited to changes in body temperature, physiological performance, free radical generation and detoxification, xenobiotic metabolism, induction of DNA damage, DNA repair, fidelity of DNA replication, control of abnormal gene expression, apoptosis, cell-cell communication, cellular proliferation, immune competency, etc.

Significance to the FDA

Fundamental to the FDA is its legislative responsibility to provide for the product safety of those items for which it has regulatory responsibility. The process of risk assessment has become integral to the establishment of safety guidelines. The assumptions used in the process of risk assessment have given greater uncertainty to such calculations than any other factor. NCTR examination of one such assumption (independency of diet) has led, and is continuing to lead, to major changes in how toxicity is evaluated and assessments conducted.

Less widely recognized and yet of equal importance is the legislative responsibility of the FDA to advise on the composition and amounts of a healthy human diet. This holistic question has been universally approached in the past in a piece-meal fashion due to the complexity and diversity of biological functions impacted by dietary intake. As a result of the program's somewhat unique, interdisciplinary approach, much of its previous work can be used to help establish what is a healthy human diet, especially in regards to caloric intake.

In many respects the efforts of this program not only cut across research areas and disciplines, as well as across agencies (NIH and FDA), but also across the overall goals of NCTR (knowledge bases, new predictive strategics' and/or method-, agent-, or concept-driven research). The project was concept based, and in order to test these concepts, new methods were developed and products patented or copywritten. The knowledge that was, and is continuing to be generated via this approach, more importantly, while adding to the

base of knowledge comprising regulatory toxicology is now being put to practical use on a daily basis by those interested in toxicology testing for regulatory purposes.

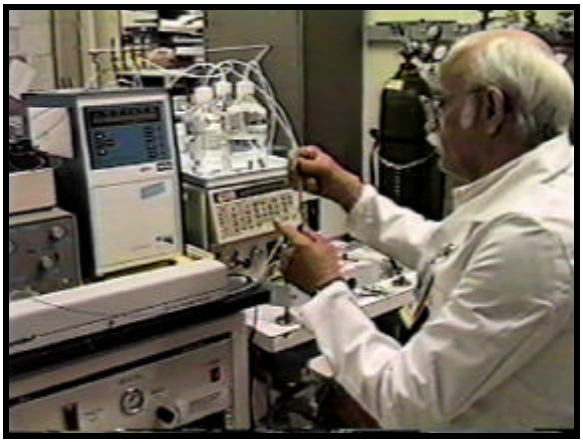
Finally, in experimental biological sciences, the ultimate test is the relevance of findings in lower animals to human health. The study initiated in FY97 in collaboration with the University of Tennessee, Memphis, will determine whether or not the findings made previously at NCTR are or are not relevant to similar events in dietary restricted humans.

CHEMISTRY

CHEMISTRY

Director: Mr. Harold C. Thompson, Jr. **TELEPHONE: 501-543-7301**

Introduction



Enforcement of regulations requires sound, reliable and validated analytical procedures as the basis of those regulations governing adulterants, contaminants, additives, as well as the composition and efficacies of FDA-regulated products. Rulings will not withstand legal scrutiny without validated analytical procedures as their basis. In recognition of this, NCTR is applying its collective expertise and equipment base in a methods development program tailored to FDA goals. Methods are being developed and validated to determine antibiotic residues in poultry, fish, beef, and milk; antimicrobial/antifungal residues in fish; and, identification of bacteria using mass spectrometry techniques. Research on development of methods and devices for efficient determination of food and seafood quality is also being conducted. The program has a strong commitment to development of analytical methods that are prerequisites for determination of test chemical purity, stability and homogeneity in the dosage form and dosage certification for chemicals scheduled for toxicological evaluation under the National Toxicology Program. These include fumonisin B_1, chloral hydrate, urethane, leuco-malachite green, malachite green, genistein and methoxychlor.

The development of analytical methods to support FDA's regulatory and enforcement actions extend beyond the scope of traditional analytical chemistry. Bioanalytical chemistry provides evidence regarding the bioactive/bioavailable form of regulated compounds, as well as evidence regarding mechanisms of action, individual susceptibility, and potential avenues to minimize the risks associated with toxicants. Research in hardware engineering provides the means to create, develop, or modify instruments needed to measure the levels of analytes of importance to FDA by more efficient means within realistic resource constraints.

The measurement, confirmation, development of hardware and the bioanalytical chemistry associated with the analysis of constituents of interest in food, drug and cosmetic products regulated by the FDA are necessary to minimize human exposure (or effects of exposure) to potentially harmful chemicals. The development of new analytical capabilities to deal with

hazards that may arise in the future indicates sound scientific planning for optimal utilization of analytical methods in protection of the public health.

The wide range of projects in this program is indicative of the diverse nature of the regulatory responsibilities of the Agency. Projects are selected based on Agency priorities and programmatic expertise.

FY 97 Goals

- Analytical Chemistry & Regulatory Analytical Chemistry: To develop both research- and regulatory-oriented analytical chemical methods for the determination and confirmation of compounds of interest to FDA/NCTR. [Method & Agent Driven Research]

- Bioanalytical Chemistry: To develop methods for the determination and confirmation of important toxicants, metabolites, biomarkers, and biopolymers of FDA/NCTR interest. [Prediction of Toxicity & Method Driven Research]

- Hardware Engineering: To create and develop instruments, interfaces, or devices that facilitate the analysis of classes of chemical and biological agents of importance to FDA/NCTR. [Knowledge Base & Method Driven Research]

FY 96 Accomplishments

Analytical & Regulatory Analytical Chemistry

The potential for rapid identification of bacteria based on spectral patterns obtained from intact whole bacteria using matrix-assisted laser desorption time-of-flight mass spectrometry (MALDI/TOF/MS) was demonstrated for the first time. Based on a small library of spectra obtained using reference bacteria, unknowns were correctly identified and distinguished from other bacteria in the library and from bacteria that had not been previously analyzed.

Pyrolysis Mass Spectrometry (PyMS) methods for characterizing analgesics by batch of origin were developed. Samples of relatively pure acetaminophen and ibuprofen were analyzed by PyMS and the data were analyzed using several statistics-based computerized pattern recognition techniques. All of the samples tested met USP specifications.

A series of studies have been conducted within the division that utilize state-of-the-art analytical and mass spectrometry techniques to identify and confirm the chemical toxicants

in FDA regulated products. A bridging study for comparison of the high pressure liquid chromatography (HPLC) and microbiological methods for determination of amoxicillin in catfish was initiated. Methods were developed for the determination and confirmation of β-agonist residues in bovine retina using liquid chromatography - atmospheric pressure chemical ionization mass spectrometry (LC-APCI/MS). These techniques were also used to quantify four heterocyclic amines in cooked meats. Multi-residue extraction and characterization techniques were developed to look at heavy metal contamination in sweeteners and to separate 14 aquaculture-related sulfonamides. The division has developed separation and detection methods for determination and confirmation of N-nitrosamines in cosmetics and nitrite cured meats, and fluoro-quinolones in livestock hair and poultry feathers. In support of NCTR/NTP chronic bioassay studies, scientists within the division are developing methods for extractions and quantitation of Fumonisin B_1, chloral hydrate, leuco-malachite green, malachite green, urethane, genistein and methoxychlor.

Applications of these methods will result in: 1) minimization of human exposure to food residues that might cause allergic hypersensitivity, drug resistant bacterial strains, and perhaps tumors or toxic effects in humans (amoxicillin, sulfa drugs, quinolones, heterocyclic amines); 2) the potential to extend the FDA confirmatory methods to drugs amenable to analysis using Atmospheric Pressure Ionization/Mass Spectrometry (API/MS) (β-agonists, sulfa drugs, quinolones); 3) assessment of human exposure to heterocyclic amines in the diet; 4) screening of sweeteners for heavy metals; 5) the development of new approaches to bacterial identification that are much more rapid than conventional techniques and hence a potential increase in sample throughput.

Bioanalytical Chemistry

The mechanism for anti-thyroid action of minocycline, a therapeutic antibiotic, was elucidated. Metabolism of minocycline by thyroid peroxidase involves the formation of reactive electrophilic species that may be important in cytotoxic, allergic drug hypersensitivity, and carcinogenesis.

The chemical compounds in soybeans that may be responsible for its goitrogenic action were identified as genistein and daidzein. The mechanisms for inhibition of thyroid hormone synthesis were elucidated and the potential for impact on humans and experimental animals was discussed.

A procedure for using Chait's peptide ladder sequence (PLS) method with unknown peptides, potentially having lysine in internal or terminal positions, was developed based on a multistep enzymatic digestion prior to the PLS steps. The technique was developed using model peptides having lysine in N-terminal, C-terminal, or internal positions.

Hardware Engineering

Several factors which affect the reproducibility of pyrolysis mass spectra (PyMS spectra) were investigated in a systematic manner. Experimental data were analyzed after normalization, autoscaling, and Fisher weighting using pattern recognition, Canonical Variates, and non-orthagonal graphical rotation. Sample mass, position, deposition, and the timing of initiation of sample pyrolysis all significantly affected spectral reproducibility. Sample electrodeposition techniques and pyrolysis timing procedures were developed to minimize the effects of these factors.

An interface received from Office of Regulatory Affairs/Los Angles Laboratory (ORA/LA) was reconstructed and installed on an existing electron impact/mass spectroscopy (EI/MS) system at ORA/NY (New York Laboratory) to provide their first LC/MS capability. This allowed liquid chromatography (LC/MS) with library searchable EI spectra to be applied to the analysis of minor components in drug raw materials manufactured overseas (with ORA's Northeast Regional Laboratory).

FY 97 Plans

Analytical & Regulatory Analytical Chemistry

Investigate use of supercritical fluid extraction/chromatography coupled with inductively coupled plasma/mass spectrometry (ICP/MS) for speciation of trace metals in seafood.

Investigate or complete development of target analyte(s) methods/multiresidue methods for the following:

- complete bridging study for HPLC vs. microbiological methods for determination of amoxicillin in catfish.

- investigate simultaneous determination and confirmatory method for erythromycin in milk, chicken, and fish using liquid chromatography/ electrochemical detection (LC/EC) and/or LC-APCI/MS.

- analyze hair from dosed cattle using LC-APCI/MS for β-agonists.

- complete evaluation of inductively coupled plasma/atomic emission spectroscopy (ICP/AES) method for Pb, Cu, Fe in cooking oils.

- complete development/validation of method for 14 sulfonamides in aquaculture species and develop LC-APCI/MS confirmatory procedure.

- develop LC/MS method for determination/confirmation of heterocyclic amines in cooked meats in collaboration with ORA Total Diet Research Center.

- prepare immunoaffinity cartridges for use in on-line sample preparation for multiresidue sulfonamide and erythromycin analysis.

- develop validated methods for analysis of N-nitrosamines in nitrite-cured meats using liquid chromatography/thermal energy analysis (LC/TEA) and LC-APCI/MS.

- develop methods using incurred tissues for determination/confirmation of new fluoroquinolones in hair and feathers.

- continue development of MALDI/TOF/MS technique for identification of bacteria.

- investigate development of LC/MS techniques for detection of staphylococcus enterotoxin.

- in the area of seafood quality, complete development of a test strip for detection of total volatile bases as an indicator of seafood quality.

- continue investigation/development of PyMS methods for bacterial identification.

- provide analytical support for toxicological evaluation of NTP compounds (FB_1, chloral hydrate, leuco-malachite green, malachite green, urethane, genistein and methoxychlor).

Completion of these activities will provide the Agency with many new methods/techniques that do not currently exist that can potentially be used in monitoring or survey programs after the methods have been through methods trails to complete their validation.

Bioanalytical Chemistry

The FY97 plans for this focal area are to:

- Determine the potential for anti-thyroid effects from inhibition of the coupling reaction in thyroid hormone synthesis. The free radical intermediates described in previous investigations suggest that this reaction will be susceptible to inhibition by a new class of compounds.

- Investigate LC/MS methodology for identification and quantification of biomarkers for goitrogen exposure in experimental animals. The suicide substrate activity of soy isoflavones and other anti-thyroid chemicals suggests that covalent peroxidase adducts are formed. Protein/peptide LC/MS methods will be used to validate this hypothesis.

- Apply LC/MS methodology to the delineation of malachite green metabolism by thyroid peroxidase and hepatic microsomal enzymes to demethylated species that may be more proximal carcinogens. The oxidation of leuco-malachite green by thyroid peroxidase is one possible mechanism for anti-thyroid effects predicted on the basis of the thyroid carcinogenicity that was observed for the closely related leuco-gentian violet. The use of proteases to enhance peptide ladder sequencing by mass spectrometry will be investigated. In particular, the potential for rapid C-terminal sequencing using immobilized enzymes specific for cleavage at this site will be studied.

Hardware Engineering

The use of chemical reaction interface mass spectrometry (CRIMS) for screening drugs by manufacturer, batch, and lot for purity will be investigated in conjunction with ORA's Northeast Regional Laboratory.

The universal interface will be used in conjunction with ORA's Northeast Regional Laboratory to develop library searchable and highly reproducible electron impact mass spectra in conjunction with liquid chromatography separation and liquid sample introduction into a mass spectrometer.

Significance to the FDA

The development and validation of relevant analytical methods will enable Agency field chemists to perform analyses of food, drug and cosmetic products for constituents that the FDA has responsibility for regulating in order to make rapid decisions on the disposition of the products requiring action.

The development of bioanalytical chemistry methods will allow the extension of analytical chemistry techniques to the analysis of complex biomolecules using analytical approaches quite different from those typically developed for more "traditional" molecules such as those produced by organic synthesis methods. In some instances, bioanalytical chemistry will provide evidence regarding the bioactive, bioavailable, or bio-altered form of a regulated compound not present in tissue residues as the expected parent compound.

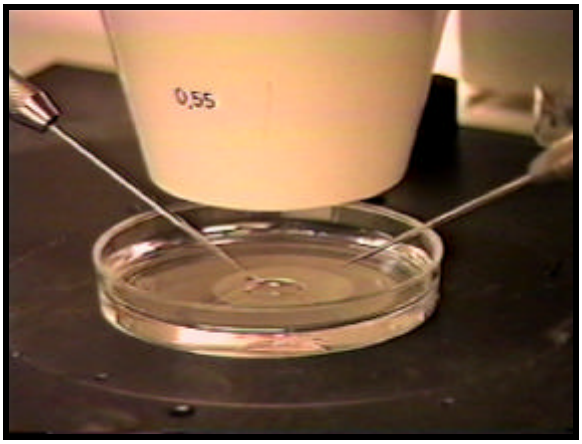
The development of specialized instrumentation hardware will allow measurements that were not previously feasible to be accomplished, or may reduce the cost of analysis significantly.

GENETIC TOXICOLOGY

GENETIC TOXICOLOGY

Director: Daniel Casciano, Ph.D. **Telephone: 501-543-7496**

Introduction



The FDA requires that petitioners provide data evaluating the potential genotoxic activity of food additives, cosmetics, and human and animal drugs they wish to market. Thus, the identification and quantitative measurement of the potency of suspected carcinogens and mutagens are essential to the FDA for regulating exposure of humans to harmful agents. Over the years, a number of *in vitro* and *in vivo* test systems have been developed to identify and quantify suspected carcinogens and mutagens. Although much information regarding certain classes of mutagenic carcinogens has accumulated, several recent studies indicate that many of these systems are insensitive to certain rodent carcinogens for which the mode of action is not readily observable. The reasons for the observations are uncertain, but are generally considered to be related to the sensitivity of the specific endpoint evaluated and the relevance of the metabolic activation system used to transform unreactive chemicals to species that react with critical cellular informational molecules leading to a genotoxic response. Alternatively, the chemical of interest may induce the carcinogenic response by indirectly damaging DNA via a secondary mechanism.

In order to increase our ability to identify potentially hazardous genotoxic and putative nongenotoxic chemicals, to understand their mode of action, and perhaps provide additional test systems when a reduced carcinogenesis protocol is employed, a variety of transgenic *in vitro* and *in vivo* mammalian systems are being developed and validated. Transgenic systems provide *in vitro* and *in vivo* tools which can more closely mimic conditions that exist in the human body, increase the potential for detecting weak carcinogens, and decrease the time required to assess accurately a chemical's capability to induce the carcinogenic process. These systems are being developed with the intent of providing relevant data which petitioners and FDA regulators may review in order to determine the potential carcinogenicity of food additives, cosmetics, and human and animal drugs.

FY 97 Goals

1. Develop and validate sensitive and predictive transgenic human and rodent *in vitro* somatic systems for the identification and quantification of human toxicants, especially carcinogens and mutagens.

2. Develop and validate sensitive and predictive transgenic rodent *in vivo* systems for the identification and quantification of human toxicants, especially carcinogens and mutagens.

The approach for the first goal is to utilize relevant *in vitro* systems to evaluate risk to the human genome. In order to accomplish this, we are using the human lymphoblastoid cell line, AHH-1, and various subclones transfected with human cytochromes P450 cDNAs for evaluation of chemicals of interest to the FDA and the NCTR. These systems are highly relevant because they are expected to mimic human metabolic activation of specific mutagens and carcinogens and to provide sensitive endpoints for assessment of cytotoxicity and mutations at both autosomal and X-chromosomal loci. Also, these systems easily lend themselves to molecular analysis making cross-species comparison possible. An additional *in vitro* system being validated is a Rat2 embryonic fibroblast cell line that carries stably-integrated copies of a lambda *lacI* shuttle vector.

The approach for the second goal is to use transgenic mice and rats to evaluate mutagens and carcinogens of interest to the FDA and the NCTR. These *in vivo* systems are expected to complement and perhaps eventually replace the present rodent bioassay because their heightened sensitivity would obviate tests at high doses where cell toxicity and mitogenicity become predominant. Also, molecular alterations can be described in the transgene, an endogenous surrogate gene, and in the cancer gene(s) allowing a more direct comparison to the molecular effects described in humans.

FY 96 Accomplishments and FY 97 Plans

There are a number of protocols ongoing in this research area. Of these, eight were initiated as a result of numerous discussions with fellow scientists and regulators from all of the FDA centers. These protocols directly address the needs stated in FY97 goals.

During the past year, we have concentrated our efforts on determining the ability of several chemicals to induce mutations and programmed cell death in the human lymphoblastoid cell lines AHH-1, h2E1v2 (expressing human CYP2E1) and MCL5, a cell line transfected with CYP1A2, CYP3A4, CYP2A6, CYP2E1 and epoxide hydrolase human cDNAs. We previously found that chloral hydrate (CH), at cytotoxic concentrations of 50% or less survival, induced

mutations both at the *hprt* and *tk* loci in the h2E1v2 cell strain but not in the non-transgenic parent CHO cell strain suggesting a requirement for CYP2E1 expression for genotoxic activity. This drug, however, mainly induced *tk* mutants indicating that it acts primarily through a clastogenic mechanism. This year this was confirmed by showing that CH and trichloric acetic acid (TCA), a metabolite, induced a dose-dependent increase in micronuclei in the CYP2E1 cell strain. A manuscript describing this effort is in preparation. This division also responded to an FDA request to evaluate micronuclei induction by phenolphthalein in MCL5.

Loss-of-function mutations in the *p53* tumor suppressor gene result in an altered response to DNA-damaging agents. Included in the mutant phenotype are the loss of cell cycle checkpoints and delayed apoptotic cell death characteristics we have consistently observed in the AHH-1 $tk^{+/-}$ cell line following exposure to DNA-damaging agents. In order to determine the functional status of *p53* gene in the AHH-1 $tk^{+/-}$ cell line, molecular analysis was performed on exons 5-9 of the *p53* gene. Initial single stand conformation polymorphism (SSCP) analysis of AHH-1 $tk^{+/-}$ revealed an abnormal migration pattern of exon 8 when compared to the control. Subsequent sequence analysis indicated that a base-pair substitution (CGG --> TGG) mutation had occurred at codon 282, a reported "hot spot" for mutations in the human *p53* gene. Neither SSCP nor sequence analysis of MCL5 indicated any difference from wild-type DNA. These results suggest that the lack of a G_1 arrest and the delayed entrance into apoptosis observed in the chemically exposed AHH-1 $tk^{+/-}$ cells are, at least partially accounted for by a loss-of-function mutation in the *p53* gene. These data are described in recent publications in Mutation Research and Environmental and Molecular Mutagenesis. Efforts are ongoing attempting to understand the signal transduction pathways responsible for triggering programmed cell death and protocols are being written to further evaluate chemicals of interest to the NCTR and FDA (such as the endocrine disrupters).

The division also has initiated approaches to goal #2 by utilizing transgenic technology to expand the number of endogenous and exogenous reporter genes suitable for detecting *in vivo* mutations. The division is utilizing rodent strains, developed by NCTR, commercially or by colleagues in other institutes, as *in vivo* systems to screen chemicals of interest to the NCTR and the FDA and to understand the processes associated with carcinogenesis. They are determining spontaneous and chemically-induced mutation frequency in the Big Blue Transgenic Rat (BlueRat) containing the *lacI* transgene, in an endogenous gene (*hprt*), and in cancer genes in target organs. Where appropriate, the mutational spectra of the reporter genes and cancer genes will be evaluated. Initial results comparing mutation frequency and phenotypic expression time of *hprt* in splenic lymphocytes are in progress. The data indicate that there is a dose- and time-dependent response in mutant frequency similar to the non-transgenic strain. However, preliminary data show the *lacI* transgene, although describing similar dose-dependent induction in mutant frequency, is following a kinetic pattern that is different from *hprt*. Two other protocols investigating the utility of the ΦX174 transgenic mouse and the p53 knockout mouse, using the newborn mouse assay paradigm as tools to

evaluate recalcitrant chemicals of interest to the FDA and the scientific community have been developed. To assist the Agency in evaluating transgenic models, the division has generated a mouse embryonic stem cell line in which one copy of the autosomal *tk* gene was disrupted by homologous recombination. This *in vitro* system has recently been shown to behave similarly to the mouse lymphoma L5178Y and AHH-1 $tk^{+/-}$ systems for evaluation of ethyl nitrosourea-induced *tk* mutants. Unlike the *hprt* locus and the existing *in vivo* transgenic loci, which mainly detect base pair substitutions, frameshifts and intragenic deletions, *in vitro* data suggest that the *tk* target will also be sensitive to mutations involving recombination and loss of heterozygosity as well as multilocus deletions. These cells are presently being used to create a mouse model heterozygous for this gene. Thus, this mouse model may provide an endogenous reporter gene that is sensitive to the major types of mutational events that are significant to human health.

Significance to the FDA

Human diseases are associated with spontaneous or induced somatic and germ cell mutations. Identification and quantitative measurement of the potency of suspected carcinogens and mutagens are essential to the FDA for regulating exposure of humans to harmful agents. These systems are capable of simulating the human condition, increasing the ability to detect weak carcinogens, and decreasing the time required to evaluate a chemical's genotoxic potential. Each FDA center has expressed an interest in and a need to utilize transgenic systems as a toxicity screen and as a model for drug/biological interaction. The development of transgenic systems can also provide a model for identifying biological activity, especially in the assessment of bioengineered products regulated by the FDA. Biotechnology product sales are expected to increase seven-fold during the next five years with over four hundred new food products alone entering the consumer market. This will greatly impact FDA's need for validated systems capable of defining exposure and assessing risk.

MICROBIOLOGY

MICROBIOLOGY

Director: Carl E. Cerniglia, Ph.D. **Telephone: 501-543-7341**

Introduction

Microbiology is an exceptionally broad discipline encompassing research areas as diverse as taxonomy, physiology, biochemistry, molecular biology, pathogenesis, food and industrial microbiology, and ecology. In fact, modern biotechnology rests upon a microbiological foundation. The microbiology research at the NCTR serves a multipurpose function with specialized expertise to perform fundamental and applied microbiology research in areas of the FDA responsibility. The microbiology research also responds to microbial surveillance and diagnostic needs for research projects within the Agency. The major aims of the microbiology research are to raise the general awareness of the importance of microorganisms in public health and to provide data to improve our understanding of the mechanisms by which toxic events occur in humans. The research is organized to handle many aspects of microbial toxicology and continue to train staff to meet the research and regulatory needs of the FDA. The microbiology research at NCTR is divided into five focal areas with strategies and objectives unique to the problem posed. Goals and accomplishments for each focal area are discussed separately below.

FY 97 Goals

1. Determine the role of intestinal microflora in the activation or detoxification of xenobiotics.

   Research on the role of gut microflora in human carcinogenesis is an important FDA need since a high proportion of human cancer is caused by environmental factors, and diet may be particularly important.

   Since the bacterial flora are in a uniquely favorable position to mediate the interaction between the gut contents and the host, it would be surprising if bacteria were not

implicated in human carcinogenesis. Therefore, the focus of this research component is: 1) to use existing models for determining the contribution of the gut microflora to foreign compound metabolism in humans and laboratory animals; 2) to relate bacterial metabolism to toxic events occurring in mammals; 3) to consider the interrelationships of bacterial and mammalian metabolic pathways; 4) to determine the effect of dietary components on the composition of the microflora in the human gastrointestinal tract; and, 5) to determine the genes involved in the metabolism and activation of pharmaceutical azo and nitrocompounds in normal populations and in patients with intestinal disorders.

Research goals for this subprogram are: 1) to delineate the metabolic potential of intestinal microorganisms and the enzyme mechanisms by which they transform drugs, azo dyes and food additives; 2) to develop additional models for assessing the risk to human health posed by exposure to synthetic and naturally occurring chemicals; and, 3) to determine the pharmacological and toxicological effects of the metabolism of chemicals such as food additives, azo compounds used as protective coating for drug delivery and prodrug azo compounds, and antimicrobial compounds on the intestinal microflora.

2. Use microorganisms as models to predict the metabolic pathways by which drugs are metabolized in mammals.

   In recent years, interest has turned to the development of alternative systems for decreasing the use of animals in laboratory studies. Microbial systems are an attractive alternative to mammalian xenobiotic and toxicity studies. The advantages are: 1) ease of experimental manipulation; 2) ease of scale-up for production of metabolites which other investigators could use for structure elucidation, biological evaluation and analytical standards; 3) lower cost; and 4) reduction of the use of laboratory animals. The focus of this research component is to develop alternative methods for toxicity testing of drugs for the FDA. This research will provide more accurate quantitative risk assessments and a better understanding of the mechanisms of toxicity.

3. Develop environmental biotechnology.

   FDA's premarket review considers potential environmental impact during the entire life cycle of a regulated product, including its manufacture, use and disposal. Under the FDA's environmental regulations, the industry sponsor of an application or petition may be required to prepare an environmental assessment of the proposed action. To support the assessment, appropriate testing of the environmental fate and effects of chemicals entering the environment may be required. The need for testing is determined by evaluation of the potential environmental exposure and the toxicity information available for a given chemical.

Due to the high cost associated with trapping, incinerating or physically removing toxic chemicals from the environment, there has been an increased interest in the use of microorganisms for the biological decontamination and detoxification of hazardous waste sites. Because the environmental risk assessment and management of potentially hazardous chemicals requires information on their occurrence, toxicity, bioavailability and persistence in the environment, we have developed multi-component environmental microcosms. These microcosms are useful for determining the rate and pathways for the environmental biodegradation of xenobiotics. The focus of this research is to isolate microorganisms which can degrade, detoxify, or accumulate hazardous chemicals and to determine the potential for their use in the bioremediation of toxic waste sites. This methodology will be used for several FDA-related research problems.

4. Develop methods for detection of contaminants

   Foodborne bacterial pathogens have been detected in contaminated foods using molecular genetic methods. Effective and sensitive methods are needed to detect contamination in foods to determine if the levels of contamination pose a public health risk. Polymerase Chain Reaction (PCR)-based methods have the potential for revealing the presence of pathogenic microorganisms in foods in a few hours while current methods require two days or longer. Rapid detection and identification of bacteria are important not only for food safety, but also for the study of the significance of the species on both *in vitro* and *in vivo* metabolic activation and detoxification of chemical toxicants and drugs and for the diagnosis of the diseases caused by these species. Development of better *in vitro* methods for rapid detection of bacterial pathogens and toxins will provide the FDA with analyses critically needed for assurance of food safety and enforcement of regulatory compliance.

5. Continue microbiological surveillance and diagnostic support of research

   Laboratory animals are susceptible to a wide variety of bacterial, viral and parasitic infections, resulting in an altered animal model that consequently affects research and testing by introducing variables that confound results. Routine screening for various infectious diseases assures reliable animal models and prevents costly, time consuming delays of research which could affect FDA regulatory decisions. Studies utilizing animals are dependent on healthy test animals; therefore, it is NCTR's responsibility to maintain the best microbiological diagnostic laboratory possible. The investigators and the FDA should be able to depend upon NCTR to support their efforts. Research goals for this subprogram are: 1) establishing and maintaining pathogen-free animals; 2) developing bacteriological assays for determining chemicals, such as folate in culture fluid, for research projects within NCTR; 3) culturing and identifying microbial contaminants for other projects and programs within the NCTR and other FDA centers; and 4) developing and testing new methods in diagnostic microbiology for other FDA centers.

FY 96 Accomplishments and FY 97 Plans

In FY96, microbiology-related research issues were discussed with microbiologists from other FDA centers and field laboratories. The scientific exchange led to the initiation of new projects and exchange of scientists between laboratories. A short summary of some resulting collaborative research projects are listed below:

I: Development of quantitative assays for measuring the tuberculocidal activity of chemical disinfectants.

Tuberculosis, once considered a disease brought under control through use of antibiotics, has re-emerged as a serious health concern in the United States. The percentage of tuberculosis cases caused by strains of *Mycobacterium tuberculosis* that are resistant to one or more of the antibiotics used in therapy is increasing. While tuberculosis is not readily transmissible by casual contact, it can be spread where individuals live or work in very crowded conditions, and perhaps by certain medical procedures as well.

Numerous chemical agents are used to disinfect and sterilize medical instruments, such as endoscopes, that cannot be autoclaved. Endoscopes contain crevices and channels that are difficult to clean and can harbor bacteria. Many of the liquid chemical germicides on the market claim the ability to kill *Mycobacterium tuberculosis*, yet improperly washed and disinfected endoscopes have been linked to the transfer of this organism from tuberculosis patients to previously uninfected individuals. This has raised the concern that some of the disinfectants may not be fully effective under the prescribed conditions.

The FDA is preparing to evaluate the tuberculocidal activity of a large number of liquid chemical germicides. The Division of Microbiology at the NCTR has been instrumental in the preparation for this evaluation by developing the expertise required to perform the Association of Official Analytical Chemists (AOAC) tuberculocidal assay, clarifying and expanding the protocol for this assay, and training ORA personnel to conduct this assay at their own facilities.

The current methods for determining the tuberculocidal activity of disinfectants are difficult to perform, poorly reproducible, and require up to 90 days to obtain a result. Scientists in the Division of Microbiology are attempting to implement molecular methods to both improve the sensitivity and accuracy of the test, and shorten the time required for a definitive answer. One such approach is the use of a mycobacteriophage carrying the firefly luciferase gene to quantitatively determine if the mycobacteria survive exposure to the disinfectants (E-6965.01). This phage should promote light production from surviving bacteria, allowing quantitation of the disinfectant activity. By developing and validating such an assay, we hope

to be able to rapidly assess the effectiveness of both currently available disinfectants and future products.

Three FDA analysts from Denver, Minneapolis, and Winchester Engineering and Analytical Center (WEAC) were trained in the AOAC tuberculocidal assay by scientists at the NCTR. The topics covered were biosafety and facility requirements, contamination control, growth and standardization of the test organism, media preparation, carrier preparation, disinfectant preparation and neutralization, phenol standardization, test performance, and interpretation of results. Discussions also included how their current facilities could be modified to provide appropriate conditions for the test.

II: Develop molecular and mass spectrometry methods for the detection of foodborne pathogens.

Despite the fact that the United States food supply is the safest in the world, tens of millions of cases of foodborne illnesses occur in the United States every year with a cost to the economy of an estimated 1 to 10 billion dollars. Therefore, the microbiological safety of food has become an important concern of consumers, industry and regulatory agencies. The U.S. Food and Drug Administration gives a high priority to protecting the public from microbial contamination of the food supply. The research program in the Division of Microbiology in FY96 has a project (E-6988) to develop molecular methods to detect and identify foodborne bacterial pathogens. In addition, scientists in the Division of Microbiology are collaborating with scientists in the Division of Chemistry to use mass spectrometry methods for the rapid identification of bacteria (E-6785 and E-6931).

A protocol (E-6988.01) for the detection of 13 species of foodborne pathogens in foods using the polymerase chain reaction (PCR) technique was developed in FY96. The method used a universal enrichment medium and the same PCR conditions with 13 sets of specific primers for the detection of foodborne pathogens. The foodborne pathogens examined were *Escherichia coli, Shigella, Salmonella, Yersinia enterocolitica, Y. pseudotuberculosis, Vibrio cholerae, V. parahaemolyticus, V. vulnificus, Listeria monocytogenes, Staphylococcus aureus* and *Bacillus cereus*. No interferences were observed using the PCR assay for food samples artificially inoculated with each single bacterial species.

In addition, a 16S rDNA-based PCR method was developed for the specific detection of *Aeromonas caviae* and *Aeromonas trota*. The detection limit was between 50 and 100 cells per gram of crab meat. This method is very rapid, obviates the need for DNA isolation from complex food matrices, and is specific for screening two pathogenic species of *Aeromonas*.

Mass spectrometric methods for the identification of bacteria were also evaluated in FY96. Scientists from the Division of Chemistry and the Division of Microbiology used pyrolysis

mass spectrometry for the rapid identification of whole bacterial cells. Suspensions of five strains of bacteria (*Escherichia coli, Bacillus sp., Pseudomonas aeruginosa, P. mendocina* and *P. putida*) were placed in wells in a Teflon cell culture plate. The cells were electrically deposited on the sample wires for pyrolysis; the mass fragments were scanned over the m/z range of 30 to 300. Pattern recognition software was used to analyze the various factors that characterized the pyrolysis mass spectra. The mass spectra obtained from 30 out of 31 different cultures of the five bacterial strains were identified correctly to the species they represented.

Another mass spectrometry method was evaluated to identify bacteria. Scientists from the Divisions of Chemistry and Microbiology used matrix-assisted laser-desorption-ionization time-of-flight (MALDI/TOF) mass spectrometry to identify intact cells of eight strains of bacteria that may be found in foods. The bacteria (*Enterobacter cloacae, Escherichia coli, Proteus mirabilis, Serratia marcescens, Shigella flexneri, Pseudomonas aeruginosa, P. mendocina*, and *P. putida*) were grown on tryptic soy agar plates, suspended in matrix solution containing α-cyano-4-hydroxycinnamic acid, and used to obtain reference MALDI/TOF mass spectra over the m/z range of 4,500 to 14,500. The mass spectra of these and other bacterial strains were analyzed for diagnostic ions that might characterize the different species. Two approaches for identification were successful: 1) comparison of new mass spectra with archived spectra from known species, and 2) co-analysis of unknown bacteria with cultures of known bacteria. Although some diagnostic ions were observed with more than one strain, enough unique ions were observed to allow all of the strains to be unambiguously distinguished from each other.

III. Assessing the effects of food additives and drugs in food on the human intestinal microflora. Determining the role of intestinal microflora in the metabolism of therapeutic drugs, food additives and cosmetics.

In recent years, questions have been raised concerning the consumption of low levels of food additives and antimicrobial residues in foods and the effect of these residues on the indigenous human intestinal microflora. Intestinal microflora are an essential component of human physiology because they act as a barrier against colonization of the gastrointestinal tract by pathogenic bacteria. They also play important roles in the digestion of food and the metabolism of drugs, xenobiotics and nutrients. Repeated exposure to antimicrobial residues and food additives may perturb the normal population density of intestinal microflora, altering enzyme activity for the metabolism of endogenous and exogenous substances, and impairing colonization resistance, which may increase susceptibility to infection by enteric pathogens such as *Salmonella*, *Shigella* and *Escherichia coli*.

The Director of the Division of Microbiology has provided guidance to scientists at the Center for Veterinary Medicine (CVM) and reviewed research protocols for the CVM on the effects

of low levels of antimicrobial residues in food on the human intestinal microflora. In addition, he, wrote a guidance document for the World Health Organization on "Assessing the effects of antimicrobial residues in food on the human intestinal microflora." This document will be used by regulatory agencies, industry drug sponsors and the international scientific community as a guideline for making an assessment of the potential risk of dietary intake of residues of antimicrobial animal drugs.

The research program in the Division of Microbiology at the NCTR in FY96 has developed molecular methods for the detection of predominant anaerobic bacteria in human and animal fecal samples. PCR procedures based on 16S rRNA gene sequences were developed and used for quantitative detection of intestinal microflora in human (adult and baby) feces and animal (rat, mouse, cat, dog, monkey and rabbit) feces. This method, including the fecal sample preparation method, is rapid and eliminates the DNA isolation steps. The method is being used in research at the NCTR for assessing the effects of food additives, antimicrobial residues and caloric restriction on the human intestinal microflora. In addition, the Division of Microbiology has been contacted by scientists from universities, pharmaceutical industry and regulatory agencies for advice and training concerning the method.

Studies have continued in the division on the determination of the role of intestinal microflora in xenobiotic metabolism. Various enzymes from the human intestinal tract play a role in the activation and/or detoxification of food additives, therapeutics, azo compounds, and nitro compounds. Some azo dyes are reduced to mutagenic compounds following reduction by bacteria from the human intestinal tract. Scientists in the division are investigating the effects of bacteria from the human intestinal tract on seven different azo dyes currently used in the food, pharmaceutical, and cosmetics industries. All of these dyes were reduced by the bacteria isolated from the human intestinal tract. Mutagenicity assays, using two strains of *Salmonella typhimurium,* showed that none of these dyes or their reduction products were mutagenic. The azoreductase genes from the various anaerobic bacteria involved in the reduction of these dyes were analyzed, and variations were found among the structures of the azoreductase genes from the different bacteria.

Azoreductase and nitroreductase convert some therapeutic azo and nitro compounds to their activated forms. These drugs are used not only for the treatment of bacterial infections but also for the treatment of inflammatory bowel diseases with unknown etiology. The reductase activities in fecal samples from pouchitis patients during the onset of the disease and following recovery was evaluated. Higher levels of azoreductase and nitroreductase were found in all of the patients following recovery. In addition, the role of anaerobic bacteria from the human intestinal microbial flora in the metabolism of nitro-substituted benzodiazepines, which are used extensively for the treatment of anxiety, was studied. These compounds have been shown to be teratogenic in experimental animals, and nitroreduction by anaerobic intestinal bacteria is considered to be involved in the mechanism of toxicity. The bacteria

isolated from the human intestinal tract that had nitroreductase activity were shown to reduce the nitrazepam to 7-aminonitrazepam.

IV. Conduct biodegradation assessments of antibiotics used in aquaculture and other priority pollutants.

Bioremediation principles, i.e., the use of microorganisms to degrade pollutants under controlled conditions to an innocuous state or to levels below concentration limits established by regulatory authorities, offers great promise for accelerated removal of chemical pollutants in the environment. A drug registration package must contain data that demonstrates that the proposed substance is efficacious against target pathogen, safe for human use and safe for the environment.

A project (E0690101) was developed in the Division of Microbiology in collaboration with the regulatory scientists of the Center for Veterinary Medicine (CVM) to evaluate the environmental impact of antibiotics and feed additives used in fish farming systems. Aquaculture industries around the world extensively use antibiotic erythromycin for control of bacterial kidney disease in salmon and trout. It is currently under review for approval in the United States. Since aquaculture waste water and sediment are discharged into the environment, there is concern over the potential detrimental effects on the environment and public health. CVM needs environmental impact and biological activity data on erythromycin for its approval.

Upon reviewing the literature, scientists in the division found that very limited studies have reported on the environmental fate of erythromycin used in aquaculture. The extensive review of antibiotics used in fish farming systems also led us to write a chapter on the environmental fate of antibiotics for an upcoming book on Bioremediation: Principles and Practice (in press). Considering the lack of available information on the environmental impact of erythromycin, the first and foremost challenge was to develop a sensitive bioassay procedure. NCTR was successful in developing a sensitive bioassay procedure to determine biological activity of erythromycin in aquaculture and environmental samples. This technique is suitable for testing water from marine and aquaculture environments, as well as extracts of a variety of environmental sediments (posters presented at the World Aquaculture meeting in Dallas and the American Society of Microbiology in New Orleans). Since then the organism *Xanthomonas* has been identified as the indicator organism (abstract submitted to the American Society of Soil Science). A manuscript describing this bioassay is presently in review for publication in an Aquaculture Journal.

The division studied the behavior of erythromycin under a variety of physicochemical and environmental conditions and found that a variety of microorganisms native to the aquaculture environment were responsible for biodegradation of erythromycin and a host of

metabolites produced lack antimicrobial activity (abstract submitted to the American Society of Soil Science). A manuscript is in preparation on the fate and degradation of erythromycin.

One of the most important aspects of this project is to develop a standard analytical method to detect erythromycin in aquaculture and environmental samples. This method once standardized, will be incorporated into CVM Environmental Assessments - Technical Methods Handbook - and used as a guide for the evaluation of drugs and feed additives requiring FDA's approval. An analytical method was developed which uses HPLC and electrochemical detector to detect and quantify trace-levels of erythromycin (manuscript in review for publication in the Journal of Food and Agriculture).

The erythromycin resistance by bacteria isolated from antibiotic-impacted sediments was evaluated and found that it is inducible and concentration dependent. Studies are in progress to purify, characterize, and identify some of the isolates. The distribution of *ErmC* gene in these isolates is being determined by DNA-DNA hybridization (abstract will be submitted to the World Aquaculture meeting in Seattle, WA).

V. Develop alternative methods for toxicity testing of drugs using microorganisms.

Because of the high costs of animal maintenance and the need to reduce animal use, alternatives or supplementary systems for animal drug metabolism are in high demand. The advantages of a microbial system as a complementary *in vitro* model for drug metabolism are low cost, ease of handling, scale-up capability and a potential to reduce use of animals. Filamentous fungi have shown the ability to metabolize drugs in a manner similar to that in mammals and are therefore potential models for mammalian drug metabolism. The goal in FY96 was to investigate further the potential of the fungal model system to produce a broad spectrum of mammalian drug metabolites and to predict mammalian drug metabolic pathways.

The research program in the Division of Microbiology in FY96 had a protocol approved (E0694200) on microbial models of mammalian metabolism. In FY96, a group of tricyclic antidepressants and antihistamines were chosen as probes to investigate their metabolic pathways by *C. elegans*. This fungus exhibits a wide range of metabolic reactions similar to mammalian livers. Fungal metabolism of cyproheptadine, amoxapine, azatadine, amitriptyline, methadilazine, chlorpromazine, cyclobenzaprine and doxepin were investigated. From the comparisons of the structure-reactivity relation of these drugs, the fungal metabolic pathways were quite similar to those found in mammalian systems including aliphatic and aromatic hydroxylation, heteroatom-demethylation, heteroatom oxidation (S,N), epoxidation, formation of dihydrodiols, as well as conjugate formation. The results of mechanistic studies for both phase I and II metabolism indicated that these reactions were catalyzed by cytochrome P450 monooxygenases similar to those found in mammals, which provided a

basis for this fungal system to be used as a predictive model for drug metabolism. These experiments also demonstrated that large quantities of metabolites, which are difficult to obtain by other means, can be easily isolated from this fungal biotransformation system.

Significance to the FDA

The Division of Microbiology seeks to continue and expand its scientific exchange and collaborative studies with colleagues at other FDA centers and field laboratories to anticipate their research needs and provide data to support regulatory activities of the Agency.

These studies include: 1) metabolism and toxicological effects of food additives, antimicrobials and macronutrients on the intestinal microflora; 2) microbial production of metabolites of mycotoxins; 3) environmental fate and effects of aquaculture chemicals; 4) tuberculocidal disinfectant testing; 5) detection of foodborne biological hazards; 6) rapid and accurate detection methods for pathogens and toxins; and 7) sensitive methods for the detection of genetically modified microorganisms.

Many of the techniques currently in use within the microbiology research area are of value to other FDA centers and field laboratories. As communication and discussion of mutual research interests between NCTR staff and other FDA scientists increases, many new projects at the forefront of applied microbiology research will be developed.

The Division of Microbiology's vision is to strive for scientific excellence and to strengthen the relevance of its research with the mission of the Food and Drug Administration. It will continue to maintain a world-class research program to solve current issues that face the FDA in the next millennium, so the Agency can make sound, science-based regulatory decisions on microbiology.

MOLECULAR EPIDEMIOLOGY

MOLECULAR EPIDEMIOLOGY

Director: Fred F. Kadlubar, Ph.D. **Telephone: 501-543-7204**

Introduction

The strategic goals of the division are: 1) the development, validation, and clinical application of molecular biomarkers of carcinogen exposure and of individual susceptibility in humans; 2) the extrapolation of results from animal mechanistic studies and animal bioassays to humans; and 3) the development and validation of "alternatives" to the standard rodent bioassay for assessment of human carcinogenicity. The intent is to better understand the mechanisms of human carcinogenesis, to provide an estimation of human exposure to genotoxic carcinogens, to assess the importance of inter-individual differences in bioactivation and detoxification toward a specific carcinogen or class of chemical carcinogens, and to suggest intervention strategies for human cancer prevention. Emphasis is on the food-borne heterocyclic amines, aromatic amines, and polycyclic aromatic hydrocarbons, and the widely used drugs, including selected benzodiazepines, antihistamines, chloral hydrate, methylphenidate, estrogens and endocrine disruptors, as well as on alcohol and tobacco usage. Projects on the etiology of human cancers of the colon/rectum, pancreas, larynx, breast, ovary, prostate, liver, lung, urinary bladder, and esophagus are either ongoing or planned for 1997.

The division's experimental approach and project areas are:

Studies to identify genetic polymorphisms that influence drug and carcinogen metabolism, individual cancer susceptibility, and therapeutic drug efficacy:

1. Food-borne heterocyclic amines and colo-rectal cancer.
    a) Chemoprevention.
    b) Metabolic polymorphisms, DNA repair, and dietary risk factors.

2. Food-borne heterocyclic amines, aromatic amines, polycyclic aromatic hydrocarbons, and pancreatic cancer.

3. Food-borne heterocyclic amines, exogenous hormone exposure and breast cancer in African-American women.
    a) Metabolic polymorphisms and dietary risk.
    b) Metabolic polymorphisms in steroid hormone metabolism.

4. Food-borne heterocyclic amines and prostate cancer in African-American men.

Human biomonitoring, DNA adduct detection, and post-market surveillance for chemical toxicants found in foods, drugs, cosmetics, and medical devices:

1. Etiology of human breast, ovarian, prostate, liver, lung, urinary bladder, and esophageal cancers.

2. Alcohol and cigarette smoking as risk factors for laryngeal cancer.

Extrapolation between animal studies and human populations:

1. Evaluation of the neonatal mouse bioassay as an alternative bioassay for selected benzodiazepines, antihistamines, chloral hydrate, methylphenidate, drugs inducing peroxisomal proliferation or oxidative stress, catechol estrogens, and endocrine disruptors including chlorinated hydrocarbon pesticides and dinitroaniline herbicides.

2. Assessment of methylphenidate hepatocarcinogenicity in non-human primates.

3. Human intervention studies with chloral hydrate and heterocyclic amines.

FY 97 Plans and Goals

During 1997, the division will continue with the projects described under the section, "1996 Accomplishments" but with increased emphasis on chemoprevention, based on recent data that the coffee constituents, kahweol and cafestol, not only induce GSTs but also appear to down-regulate NAT2. In animal studies that will require further validation, the division has effectively changed the phenotype of rats from rapid to slow acetylators. A pilot study is also being planned in humans, using coffee prepared with and without the use of filter paper, which quantitatively retains these terpenoids. These results could have a profound impact on our ability to predict adverse drug reactions and the impact of coffee consumption on colon and bladder cancer risk.

Similarly, further attention will be placed on the regulation of CYP1A2 gene expression, given the importance of this enzyme as a determinant of therapeutic drug efficacy and cancer

susceptibility. The mechanisms that control gene expression will be explored, including analysis of genetic variants as well as changes in gene methylation, an epigenetic mechanism. The *CYP1A2* genotype and the methylation profile of the *CYP1A2* gene will be determined in human liver tissues grouped according to age, gender, and smoking status. Methyltransferase and SAM/SAH levels will also be determined. Because CYP1A2 is also a major metabolizing enzyme for aflatoxin B_1, these efforts will subsequently lead to NCTR participation in an ongoing case-control study (in collaboration with the NCI) being conducted on aflatoxin B_1-induced liver cancer patients including both hepatitis B-positive and -negative individuals. At the same time, these samples will be utilized to examine the biological significance of a genetic polymorphism in *CYP2E1*, by comparing genotype to expressed levels of the enzyme in human liver microsomes. Recently in collaboration with a visiting scientist and Chief of the Molecular Epidemiology Division at the National Cancer Institute in Beijing, division research has provided the first evidence that this polymorphism in *CYP2E1* is a strong risk factor for esophageal cancer in China (Linxian County), where food-borne nitrosamines known to be bioactivated by CYP2E1 are strongly implicated in the etiology of this cancer.

The division will also focus its attention on analysis of molecular epidemiological data from its case/control study on pancreatic cancer which, like colo-rectal cancer, shares common risk factors that suggest the role of food-borne heterocyclic amines, including high meat and fat consumption and low intake of cruciferous vegetables and fruits. Furthermore, pancreatic cancer risk is further increased by cigarette smoking and certain occupations, which suggests the involvement of carcinogenic aromatic amines or polycyclic aromatic hydrocarbons (PAH's) as etiologic agents. Initial studies in this laboratory on the metabolic activation of aromatic/heterocyclic amines by human pancreas tissues indicate that both NAT1 and NAT2 are expressed, albeit at lower levels than in the colon: while CYP1A2 and other CYP's involved in carcinogen metabolism are not detectable in smokers or non-smokers. Preliminary experiments on the analyses of putative carcinogen-DNA adducts in human pancreas by ^{32}P-postlabelling have also suggested the presence of both aromatic amine and PAH adducts; but only the latter group were elevated in cigarette smokers. Efforts are currently underway to confirm the identity of carcinogen-DNA adducts present by immunochemical and S^{35}-postlabelling techniques [in collaboration with Columbia University and MIT]. This study is expected to provide an assessment of the relative roles of dietary and environmental carcinogens in human pancreatic cancer and to result in appropriate recommendations for protecting public health.

In preliminary studies, the division has obtained evidence that the heterocyclic amine, 2-amino-α-carboline (AαC), which is found in cooked foods and is the predominant aromatic amine carcinogen in cigarette smoke, is present as a major DNA adduct in human larnyx. During 1997, they plan to complete characterization of this DNA adduct and develop an LC/MS method [with in the Division of Chemistry at NCTR] to confirm adduct levels in human tissues. In addition, we have data that indicate that acetaldehyde-DNA adducts may be

formed *in vivo* after treatment with [^{3}H]ethanol. Recently, others have shown that acetaldehyde-modified DNA can be analyzed by ^{32}P-postlabelling (after reduction to ethylated adducts). Acetaldehyde has been shown to be carcinogenic to rodents after inhalation and it induces chromosomal damage but not mutations in a variety of *in vitro* tests. On the other hand, ethanol has not been shown to be carcinogenic or mutagenic in experimental systems; however, both acetaldehyde and ethanol have been reported to be co-carcinogens (*e.g.,* with benzo[a]pyrene, diethylnitrosamine, or vinyl chloride) in different animal models. Human epidemiological studies are also consistent with ethanol as a co-carcinogen, particularly when combined with tobacco usage. Of these, the relative risk for cancers of the upper aerodigestive tract, especially the larynx, show the most consistent synergism between total alcohol intake and heavy cigarette smoking. Thus, the division proposes to examine the hypothesis that ethanol forms acetaldehyde-DNA adducts in human larynx and that these adducts may serve to enhance the relative persistence or mutagenic outcomes of AαC and other smoking-related DNA adducts.

Another major emphasis in 1997 will be directed at the possible role of chemical carcinogens in breast cancer etiology, and the modification of risk associated with these exposures by polymorphisms in genes involved in carcinogen biotransformation. Because breast cancer most commonly arises from ductal epithelial cells, in 1996 the division began a study to examine those cells shed into human breast milk for carcinogen-DNA adducts. They have been obtaining specimens from nursing mothers, both smoking and non-smoking, and have developed methodology to separate exfoliated ductal epithelial cells from human breast milk. In the coming year, they will continue with extraction and postlabeling of DNA for the presence and characterization of carcinogen-DNA adducts in human breast epithelial cells. Variability in adduct levels related to both exposure and to genetic susceptibility based on variability in carcinogen metabolism is expected. A study of chemical carcinogenesis in human breast epithelial cell studies, and of the effects of environmental and drug exposures and genetic susceptibility on these processes, should have important implications for future regulatory decisions. By sorting out etiologic mechanisms and putative risk factors in breast carcinogenesis, subgroups of individuals susceptible to specific carcinogens, particularly heterocyclic and aromatic amines, may be identified.

The division is also beginning case-control molecular epidemiologic studies of breast and prostate cancer in African-American women and men. Because African-American men have the highest incidence of prostate cancer in the world, and African-American women have twice the risk of Caucasians of premenopausal breast cancer, the NCTR is interested in evaluating possible genetic and environmental factors that may account for these racial disparities. These include dietary factors, particularly consumption of dietary, heterocyclic amines, hormonal factors (oral contraceptives, hormone replacement therapy and reproductive factors), and genetic variability in the metabolism of heterocyclic amines and steroid hormones. These hypotheses will be applied to both breast and prostate cancer. Extensive questionnaire data, as well as blood specimens and urine for caffeine phenotyping

will be collected. A nested case-series study is also planned to identify and characterize DNA adducts in prostate tissue from men who are participating in the study. As in the study of exfoliated ductal epithelial cells in human breast milk, the division will evaluate levels of adducts in relationship to environmental exposures, as well as to polymorphisms in genes involved in metabolism of dietary and environmental carcinogens and endogenous steroid hormones. They are also planning to evaluate loss of expression of *kang ai 1*, a putative anti-metastases protein, in relation to stage and grade at diagnosis, levels of DNA adducts and environmental exposures, in collaboration with the NIEHS.

Because of the racial disparities in breast and prostate cancer incidence, and the likelihood that diet may play a profound role in the etiology of both diseases, the division is beginning a survey of dietary habits of rural African-American men and women in the Mississippi River Delta region in eastern Arkansas. There is little information regarding eating habits of rural African-Americans in the southern United States, and it is questionable if existing food-frequency questionnaires are relevant for these populations. Using 24-hour diet recalls, researchers hope to obtain detailed dietary information, from which they can evaluate the utility of existing survey instruments. These data may be extremely important for future studies on the role of diet in disease etiology in African-Americans, and may have major implications for FDA monitoring of food-borne carcinogens.

The division is also beginning to focus on the role of metabolism of steroid hormones in carcinogenesis in hormonally responsive tissues. This includes a study of estrogen metabolism in human ovarian tissues and of the effects of hormonal regulation in combination with genetic variability on these processes. Identification and characterization of estrogen-specific biotransformation pathways in normal tissues will enable the dissection of events that participate in the generation of, and/or the protection from, the production of highly estrogenic or DNA-damaging metabolites. Specifically, the study will: 1) identify the major metabolic enzymes acting on estradiol in the human ovary as a function of hormonal exposure associated with ovulation; 2) characterize metabolic phenotypes for these major enzymatic activities (*e.g.*, high, intermediate and low); 3) identify variability in metabolic activity as a function of hormonal exposure and/or individual variability; and 4) determine the molecular mechanisms that account for any observed phenotypic variability. It is predicted that these studies will identify estrogen-specific enzyme isoforms expressed in the ovary that demonstrate significant hormonal responsiveness and/or interindividual variability. These studies have the potential to aid in the definition of a group of individuals at greater risk from estrogen exposures for developing hormonally induced cancers.

Other areas of research that are still in the early stages involve similar molecular biomarkers studies, with special emphasis on the role of CYP1B1 in steroid hormone and in carcinogen/drug metabolism. The division has thus far prepared anti-peptide antibodies and have established functional assays for CYP1B1, and is now planning immunohistochemical studies on tissue localization in humans. They have also set up the host reactivation DNA

repair assays, using aromatic and heterocyclic amine-adducted DNAs, that will allow examiniation of interindividual differences in DNA repair for carcinogen adducts that have been specifically implicated in human cancer etiology.

In addition to the continuation of its efforts on validation of the neonatal mouse bioassay, the division will propose, in collaboration with the Division of Neurotoxicology, an addendum to an existing three-year protocol that has been examining the effects of methylphenidate (Ritalin) on cognitive function in young Rhesus monkeys. The dose range and blood levels used are similar to that used to treat narcolepsy in adults and more widely, to treat attention-deficit disorders (hyperactivity) in children. A continuation of this dosing for an additional three years should allow an assessment of any neoplastic changes in the dosed animals. There is currently a high level of concern within CDER due to an NTP report that found hepatoblastomas in rats (5/50) given high doses of methylphenidate. Since this tumor is extremely rare in rats, these findings must be carefully considered, particularly since the most common childhood liver tumor, albeit rare, is also hepatoblastoma.

FY 96 Accomplishments

During 1996, division studies on genetic polymorphisms were focused on the bioactivation and detoxification of the food-borne heterocyclic amines, which have been of increasing public health concern to FDA. Using animal models, human tissues, and molecular biomarkers in epidemiological studies, the bioactivation of heterocyclic amines to colon carcinogens in humans was found to involve *N*–oxidation followed by *O*–acetylation to form the *N*-acetoxy arylamine that binds to DNA to form carcinogen–DNA adducts. These steps are catalyzed by the hepatic enzymes, cytochrome P4501A2 (CYP1A2) and acetyltransferase-2 (NAT2), respectively, which NCTR and others have shown to be expressed polymorphically in humans. The division has recently found four variant alleles in the CYP1A2 gene and now have evidence for a genetic polymorphism that is associated with CYP1A2 inducibility. It is believed that this finding will have a major impact not only on cancer susceptibility, but also on therapeutic drug efficacy and hormonal interactions, since CYP1A2 is also the major enzyme metabolizing many drugs and estrogens. Thus far, research has shown that, consistent with the proposed metabolic activation pathway for heterocyclic amine carcinogens, subjects at greatest risk for colo-rectal cancer or non-familial polyps are those who possess both the rapid NAT2 genotype/phenotype and the rapid CYP1A2 phenotype and who are exposed to high dietary levels of carcinogenic heterocyclic amines. Moreover, a logistic regression model that included both metabolic genotypes/phenotypes and consumption of well-done red meat suggests that, in terms of attributable risk, these susceptibility factors together with food-borne heterocyclic amine carcinogen intake may account for about half of the sporadic colo-rectal tumor incidence observed in the U.S. At the same time, research has found that

glucuronyl transferases (UGTs) and the α-class glutathione *S*-transferases (GST), human GST A1 and rat GST 1a,1b, can effectively detoxify the heterocyclic amines. This finding is of particular significance since UGTs and α-class GSTs are known to be inducible in humans and experimental animals by consumption of cruciferous and yellow-green vegetables; and the latter has been shown by epidemiological studies to be one of the most consistent protective factors against human colo-rectal cancer.

Since colo-rectal cancer is the most prevalent cancer in non-smokers in the U.S., appropriate intervention strategies and concomitant public health recommendations are of paramount importance. To determine the potential for dietary intervention in modulating the carcinogenicity of the heterocyclic amines, the effect of a variety of treatment regimens on the bioactivation and detoxification pathways was examined. The paradigms used are representative of classes of dietary substances associated with chemoprevention in humans and have been shown to affect tumorigenesis in animal models. These included dietary tannic acid, Oltipraz, α-angelicalactone, quercetin, ethoxyquin, indole-3-carbinol, diallyl sulfide, benzylisothiocyanate, kahweol:cafestol (1:1), black tea, green tea, high dietary fiber, and physical exercise. Of these, consumption of black tea and dietary kahweol:cafestol (the major terpenoids in coffee) had the strongest effect on inhibiting heterocyclic amine-DNA adduct formation in the rat colon model. The mechanisms of action of these agents differed and appear to involve potent inhibition of CYP1A2 by black tea and induction of α-class GSTs by kahweol:cafestol, suggesting the potential for combined use in dietary intervention studies.

In the area of human biomonitoring and DNA adduct detection, similar progress has been made in understanding the etiology of urinary bladder cancer, where occupational exposures and cigarette smoking are regarded as major risk factors. NCTR research had previously provided strong evidence, based on metabolic polymorphisms and DNA adduct detection, that smoking-related aromatic amines are major risk factors. However, other epidemiological studies have suggested that exposures to polycyclic aromatic hydrocarbons (PAHs) from smoking, occupational or environmental sources may also increase bladder cancer risk and could account for a substantial proportion of bladder DNA adducts. Recently, the division has found that PAH-DNA adduct levels are correlated significantly with the ability of the bladder to bioactivate benzo[*a*]pyrene (BP) and have identified the activating enzyme as lipoxygenase. In addition, genotyping studies have suggested that glutathione *S*-transferase (GST) M1 may play an important role in the detoxification of PAH bladder carcinogens, since individuals with the *GST M1* deletion are at higher risk for bladder cancer. Thus, we have also examined the relationship between adduct levels and *GST M1* genotype and the expression of GST enzymes in the bladder epithelium. However, there was no effect of the *GST M1* genotype on DNA adduct levels and the predominant GST ($\approx$ 90%) in bladder cytosol was found to be GST P1. Interestingly, it was found that the levels of this enzyme in the bladder appear to be expressed in a polymorphic manner and are inversely related to PAH-DNA adduct levels in this tissue. Moreover, individuals who are both rapid metabolizers

for BP and possess low GST P1 activity have four to six-fold fold higher bladder PAH-DNA adduct levels than poor BP metabolizers with the high GST P1 activity phenotype.

This effort subsequently led to a collaboration with the University of Dundee and resulted in the identification of a genetic polymorphism in GST P1, which involves an amino acid change at the active site of the protein and thus alters substrate activity. Since this GST is the major detoxifying enzyme in human extrahepatic tissues and is up-regulated in human tumor tissues, it is expected that this polymorphism will play a critical role in both cancer susceptibility and in chemotherapeutic drug efficacy. Accordingly, collaborative studies have begun to test these hypotheses with the National Institute of Public Health in Budapest, with the NCI, and with the UAMS.

Another project involving the use of molecular biomarkers in a pancreas cancer case-control study is nearly completed with some 100 cases and 300 controls entered into the study.

International collaborative efforts in the area of human DNA adduct biomonitoring have also been undertaken by the division, together with the NCTR Division of Biochemical Toxicology, the U.S. EPA, and the International Agency for Research on Cancer. This working group has organized and are participating in interlaboratory trials for the detection of carcinogen-DNA adducts in humans and its application to human risk assessment. This effort now involves some 30 laboratories world-wide and is expected to form the basis for the use of DNA adduct measurements in making regulatory decisions. As part of NCTR's commitment to research progress in this area, the Division served as a principal organizer and sponsor of the 6th International Conference on Carcinogenic and Mutagenic Aromatic and Heterocyclic Amines (Monterey, CA) and the International Conference on DNA Adducts and Mutations in Human Biomonitoring (Stockholm).

Projects involving the extrapolation between animal models and humans have thus far focused primarily on the validation of the neonatal mouse bioassay as an alternative model for identifying genotoxic carcinogens. The evaluation of several widely used benzodiazepine and antihistamine drugs, as well as chloral hydrate, methylphenidate, drugs inducing peroxisomal proliferation or oxidative stress, catechol estrogens, and endocrine disruptors, including chlorinated hydrocarbon pesticides and dinitroaniline herbicides, are ongoing in the neonatal mouse and are being compared to studies being conducted by the National Institute for Environmental Health Sciences (NIEHS) on other alternative rodent bioassays. The compounds selected represent major classes of drugs that are widely used in human populations. A common concern for many of these compounds arises from drug-related increases in the incidence of mouse livertumorsobserved in standard two-year carcinogenicity studies. In this regard, the mechanism of tumor induction is unclear and both genotoxic and nongenotoxic processes have been proposed. However, in the neonatal mouse tumorigenicity bioassay, only two doses of the test compound, given to preweanling animals, are required to obtain positive results after 12 months; and, thus far, only genotoxic

carcinogens have been shown to be active in this test system. Therefore, we believe that this bioassay, when combined with relevant mechanistic information in human cells and in human epidemiological studies, will provide a more definitive assessment of the significance of marginal findings in the standard rodent bioassay and will also become a useful supplemental or alternate carcinogenicity screening method for FDA-regulated drugs or drug products.

Significance to the FDA

These research projects are being carried out to identify human polymorphisms in carcinogen and drug metabolism and to provide direct evidence for human exposure to specific chemical carcinogens. Furthermore, correlational analyses between DNA adduct levels and carcinogen-metabolizing enzymes in the same individuals allows not only the identification of populations who may be at higher risk for chemically-induced cancers but also provides evidence for the role of different chemical classes in human cancer etiology. Together, these efforts are expected to result in better public health monitoring and regulatory risk assessment of food and drug carcinogens and in appropriate strategies for earlier disease diagnosis and cancer prevention.

NEUROTOXICOLOGY

NEUROTOXICOLOGY

Director: William Slikker, Jr., Ph.D. **Telephone: 501-543-7203**

Introduction

The Congressional designation of the 1990's as the Decade of the Brain underscores the tremendous opportunities offered by the current and anticipated advances in brain research and the enormous cost of mental disorders to the national economy. In the United States, brain-related disorders account for more hospitalizations than any other major disease group, including cancer or cardiovascular diseases. One out of four Americans will suffer from a brain-related disorder at some point in their life-time, and the cost to the national economy for treatment, rehabilitation and related consequences is an estimated $400 billion each year. At no time in the past, however, have researchers been better poised to gain understanding of brain-related disorders and to reduce risks associated with neurotoxicity.

According to the Congressional Office of Technology Assessment's recent report on neurotoxicity, the known or suspected causes of brain-related disorders include exposures to chemicals such as therapeutic drugs, food additives, foods, cosmetic ingredients, pesticides and naturally occurring substances. The number of potential neurotoxicants that will require FDA regulation has been estimated to be in the thousands and yet guidelines for neurotoxicity risk assessment remain vague and underdeveloped compared to those for cancer. Chemicals such as those listed above are also vital to the national economy and our daily lives are markedly improved by them. The problem is to determine at what dose and under what conditions a specific chemical may produce nervous system-related toxicity.

FY 97 Goals

The overall goals of neurotoxicology are to develop and validate quantitative biomarkers of neurotoxicity and to utilize them to elucidate toxic mechanisms. This will increase the certainty of assumptions underlying risk assessment for neurotoxicants. The strategy for achieving these goals has been to develop a multidisciplinary approach that integrates neurochemical, neuropathological, neurophysiological, and behavioral

assessments to determine effects and mechanisms of neurotoxicity. The unique features of the neurotoxicology research efforts at NCTR include the capability to determine target tissue concentrations and cellular interactions of neurotoxicants, and to reduce the uncertainty of extrapolating data across species by effectively using rodent and nonhuman primate animal models as well as humans whenever possible.

Over the last decade, expertise, equipment and facilities have been woven together to pursue the overall goals of neurotoxicology research through five primary objectives or focal research areas. These focal areas have been developed based on prevailing scientific understanding and on the importance of each area to regulatory concerns and include: 1) excitatory amino acids as mediators of age and neuroanatomical susceptibility to neurotoxicants; 2) the role of aromatic monoamines in neurotoxicity; 3) disrupters of energy metabolism and axonal transport; 4) oxidative-stress-induced neurotoxicity; and 5) interspecies extrapolation and validation of animal models. Recently, a sixth focal area has been added entitled neurohistochemical development and validation. These focal areas represent mechanistically based approaches for defining and understanding the potential for a broad range of drugs and other chemicals to produce neurotoxic effects. In some instances, either chemicals or age (development or senescence) may be used as tools to better understand the pathogenesis of neurotoxicants.

FY 96 Accomplishments

The interdisciplinary approach, the use of multiple, established animal models and innovative biomarkers, and an in-depth working knowledge of and experience with mechanistically-based focal areas of research enable the neurotoxicology's research group to be responsive to FDA regulatory needs. There are several ongoing or planned studies, many in conjunction with other FDA centers, that exemplify the application of neurotoxicology research's approach to providing critical research information applicable to FDA's regulatory problems. The seafood neurotoxicant, domoic acid, and the prototypical excitotoxicant, kainic acid, are being evaluated as part of the excitatory amino acid focal research area in conjunction with colleagues at CFSAN and CDER. Progress to date includes the development and validation of neurochemical, neuropathological and behavioral methods for assessing alterations in amino acid neurotransmitters, dopamine release and specific neurohistological and behavioral indices associated with the N-methyl-D-asparte (NMDA)/glutamate receptor system. Several publications describe the dose response and age relationship of domoic acid exposure and the resultant lesions in the hippocampus and other brain areas in the monkey, and neurohistological and behavioral alterations in the rat. These studies have been extended to examine the potential for developmental effects and to allow for the application of quantitative risk assessment procedures.

Methods for assessing the neurotoxicity of the anorectic agent, d-fenfluramine, have been developed during the comprehensive study of methylenedioxymethamphetamine (MDMA) and methamphetamine (METH) under the monoamine focal area of research. These and other positive controls have been used to develop and validate the use of neurochemical monoamine concentrations, monoamine and excitatory amino acid release and receptor characterization, neuropathological (nerve terminal degeneration), and behavioral (spontaneous and operant) procedures for the quantitative assessment of the monoamine neurotransmitter systems. Furthermore, these data, and data on the influence of environmental temperatures and pharmacodynamics on neurotoxicity, have enabled a description of more defined mechanistic pathway through which the neurotoxicity of substituted amphetamines produce neurotoxicity. Recently, rodent studies have demonstrated that core body temperature is a major determinant of the influence of d-fenfluramine on the serotonergic system in the brain. Data generated from multiple species exposed to a variety of doses of MDMA have been used to develop a biologically based, dose-response model for the quantitative risk assessment of neurotoxicants. This model, which allows the use of continuous data, is one of a handful of examples used by recent review committees (*e.g.*, the National Research Council, International Life Sciences Institute [ILSI]) to exemplify quantitation of the risk assessment process for neurotoxicants.

The multispecies neurotoxicological assessments of several anti-HIV agents (*e.g.*, dideoxycytidine [ddC] and dideoxyinosine [ddI]) and the anti-tuberculosis agent isoniazid, in conjunction with colleagues at CDER and the National Institute of Environmental Health Sciences (NIEHS), are currently in progress under the axonal transport/energy disruption focal research area. Neurophysiological (nerve conduction studies), behavioral (operant and spontaneous) and histological (glial fibrillary acidic protein [GFAP], immunocytochemistry, degeneration-specific stains and c-fos activation) methods have been developed to assess the effects of energy disruptors/transport inhibitors. A recent manuscript describes the novel findings that chronic ddC exposure in the monkey results in specific lymphocyte cell surface marker alterations in a dose-related manner. Another recently submitted manuscript describes the first animal model of ddI-induced peripheral neuropathy and the first rodent model of isoniazid-induced demyelination of the central nervous system.

In cooperation with colleagues at CFSAN, the essential trace metal manganese is being evaluated with techniques developed for trimethyltin and methylmercury under the oxidative stress focal research area. The relationship between organometal-induced neurotoxicity (*e.g.,* methylmercury, triethyllead, trimethyltin) and oxidative stress has been examined with the newly developed *in vitro/in vivo* probe dichlorofluorescein. Generation of free radicals during oxidative stress has been correlated with lipid peroxidation, superoxide dismutase (SOD) transgenic alteration, changes in neurotransmitter receptor binding and alterations in cellular activity at the molecular level (c-fos, heat shock proteins). These techniques were also applied to other selective neurotoxicants such as 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) and will be utilized along with neurohistological methods and

behavioral assessments of memory and learning. Progress has also been made on the oxidative-stress-producing potential of manganese and the importance of metal valence on neurotoxicity potency.

The importance of developing appropriate animal models for use in interspecies comparisons of the effects of neuroactive agents has led to the development of automated systems for administering several complex behavioral tasks to laboratory animals as well as humans. These tasks are usually identical or very similar for all species. The maintenance of task continuity across species allows for the quantitative determination of similarities and differences in complex brain function and assists in the extrapolation of data from laboratory animals to humans. Additionally, the recent demonstration that performance of several of these tasks correlates significantly with IQ in humans, serves to validate their use in studying important aspects of brain function in animals. In addition to the development and validation of the above-mentioned biomarkers of effect in the normal adult animal, the modulation of neurotoxicological outcome by age (development and senescence), nutritional status and body temperature have been frequently examined. The neurotoxicology research staff have enhanced scientific exchange by serving on several interagency committees as FDA/NCTR representatives. These committees include the Interagency Committee on Neurotoxicology, the FDA Intercenter Neurobiology/Neurotoxicology Working Group, ILSI Working Group on Human Variability and "Red Book II" revision. In addition, members of the staff have co-organized several national or international conferences such as the annual meeting of the Behavioral Toxicology Society and the "Third International Conference on Neuroprotective Agents" and "Cellular and Molecular Mechanisms of Drugs of Abuse" which resulted in published, peer-reviewed proceedings. These conferences have brought together scientists from government, industry and academia for information exchange and consensus building concerning methods development and risk assessment procedures for neurotoxicants.

FY 97 Plans

Several research projects in the various focal research areas are scheduled for initiation in FY97. Within the excitatory amino acid area, domoic acid-induced effects will be evaluated in the developing rat (collaboration with CFSAN). In addition, after the recent publication of a new sensitive and reliable fluorescent method for revealing neuronal degeneration and a simple, sensitive and reliable metallic stain for demonstrating myelin pathologies, a new research focal area on neurohistological technique validation has been initiated. In the monoamine focal area, the influence of body temperature on d-fenfluramine induced neurotoxicity will be further explored and studies on this and other stimulants (*e.g.*, methylphenidate) will be completed (collaboration with CDER). For the energy disruption focal area, data demonstrating the utility of animal models for the study of anti-HIV

therapeutics (*e.g.*, ddI and ddC) will be published (collaboration with NIEHS and CDER). Also, methods for detecting brain levels of d-fenfluramine and its active metabolite will be published to improve risk assessment procedures for this agent. The time to onset of the histologically verified peripheral neuropathy induced by ddI will also be resolved. Two new protocols, one to examine the fetal disposition of 3'-azido-3'-deoxythymidine (AZT) and dideoxy-didehydrothymidine (d4T), and another to evaluate the monkey as a model to study the peripheral neuropathy-producing effects of thalidomide and ddC, will be completed in collaboration with NIEHS and CDER. In the oxidative stress focal area, studies of the effects of manganese on the nervous system in the adult and developing rat will be completed and published (collaboration with CFSAN). A recently approved protocol that focuses on the neurotoxicity potential of Ibogaine will be initiated. In collaboration with CFSAN, 3-nitropropionic acid (3-NPA), a food-borne agent known to produce mitochondrial dysfunction, will be used in an attempt to develop a chemically induced rat model of "ischemic hypoxia." In the interspecies extrapolation and validation of animal models focal area, validation studies on the acute effects of representative drugs in the NCTR operant test battery will continue in the monkey and rat as will studies on the chronic effects of the prototypic drugs *(e.g.*, methylphenidate) used in the treatment of attention deficit and hyperactivity disorder (ADHD). Results on the use of the NCTR operant test battery for the assessment of normal and ADHD children will be published.

New areas of effort within the neurotoxicology research group follow along the lines of the NCTR Science Advisory Board (SAB) Neurotoxicology Subcommittee recommendations and include: 1) the development of a neurotoxicology cell culture facility to investigate the neurotoxic potential of the fumonisins (CFSAN collaboration) and other FDA-relevant agents; 2) the development of molecular biology techniques to detect and describe the effects of aromatic monoamines (*e.g*., methamphetamine, fenfluramine, methylphenidate) on neurotrophic factors and agents postulated to induce oxidative stress (amphetamines, metals and MPTP); and 3) the completion of the electrophysiology laboratory to continue the studies on 3-NPA and domoic acid neurotoxicity and aid in the risk assessment of these agents.

Development of neurotoxicological knowledge bases are an integral component of the overall scheme to derive predictive values for human risk. Knowledge bases are accumulations of data that have predictive values that reliably extend beyond individual data elements within a database. Predictive capabilities are achieved through the application of artificial intelligence programs such as neural networks, machine learning, expert systems, or other approaches currently being used and developed. The foundation of knowledge bases consist of biological endpoints (*e.g.*, neuropathological, neurophysiological, neurochemical, molecular biological and behavioral), data concerning mechanisms of action, structure activity relationships (SAR), target tissue concentrations, and physical/chemical properties of the agent. Hence, the prediction of human risk can be derived from the working model by assembling information in an ascending order of complexity from method-, agent-, or concept-driven research to strategies for prediction (*e.g*., SAR and species extrapolation models) to

databases. A complete database can be envisioned as the product of interactive and iterative processes between the several foundation components (*e.g.*, endpoints and mechanisms). In the process of developing knowledge bases from various data sources via quantitative risk assessment procedures, deficits in existing data will be identified that will determine directions for new research priorities. Subsequent studies can then be conducted to fill these identified data gaps to help complete the knowledge base.

In addition, the Proceedings of the "Third International Conference on Neuroprotective Agents," (supported and organized by members of the Division of Neurotoxicology) and consensus-building documents concerning the neurotoxicity assessment of food contaminants and other FDA-regulated agents (CFSAN collaboration) will be published.

Significance to the FDA

The importance of the interdisciplinary mechanistically-based approach of neurotoxicology research is that it encourages the development of in-depth, integrated knowledge bases and techniques that will be useful in addressing problems associated with current (*e.g.*, thalidomide, fumonisin [FB_1], domoic acid, methylphenidate, fenfluramine, ibogaine, and fluoxetine) and future agents of regulatory concern.

As stated in the recent Office of Technology Assessment (OTA) document on neurotoxicity, NCTR has the facilities, equipment and personnel to expand interdisciplinary research in neurotoxicity and to conduct research related to therapeutic drugs and food additives. Although neurotoxicology research at NCTR currently represents a major portion of FDA's neurotoxicology efforts, it must maintain its flexibility in order to deal effectively with future FDA needs. The following four-fold plan has been developed to allow neurotoxicology research to keep pace with FDA's responsibility to assure safe and effective drugs, foods, devices, and cosmetics. First, we must continue and enhance interactions with other FDA centers in order to better understand and address FDA regulatory concerns. Second, we need to expand efforts in interdisciplinary and fundamental research approaches, especially in the molecular and interspecies areas, in order to validate appropriate animal models and quantitative risk assessment techniques for neurotoxicants. Third, we need to continue to develop and validate improved quantitative risk assessment procedures with broad applicability, and fourth, we need to continue to develop predictive system and knowledge base approaches to solve neurotoxicological problems. Integration of neurotoxicology research, FDA-wide, will provide the scientific basis necessary for sound regulatory decisions.

REPRODUCTION & DEVELOPMENTAL TOXICOLOGY

REPRODUCTIVE & DEVELOPMENTAL TOXICOLOGY

Acting Director: B.A. Schwetz, D.V.M., Ph.D. **Telephone: 501-543-7517**

Introduction

Increasing recognition of the importance of women's health issues reemphasizes the need for better identification of developmental toxicants and improved assessment of their risk. Congenital malformations recognized at birth affect one in 14 infants (7%); this doubles when later-recognized deficits are included. Some experts estimate that at least one child in three has a birth defect. Additionally, another 7% of infants have low birth weights and at least 25% of recognized pregnancies end in spontaneous abortion.

Birth defects cause over 20% of all infant deaths and are the fifth leading cause of potential years of life lost. More money is spent by states on developmental disabilities (including mental retardation) than on any other category of chronic disease. Over one dozen chemicals, the majority of which are FDA-regulated, are recognized as human teratogens; many more agents are suspected human teratogens. However, no chemical regulated by FDA has been tested for developmental toxicity in pregnant women; only recently have non-pregnant women been included in clinical trials, and some consideration is now being given to also including pregnant women. This puts a heavy burden on laboratory animal research.

FY 97 Goals

1. Develop improved methods and new strategies for detection and prediction of developmental toxicity in laboratory animals and the human population, focusing on reproductive tract development, central nervous system development, whole embryo development, pharmacokinetics during development, and the molecular biology aspects of development.

2. Develop new concepts on how xenobiotics produce developmental toxicant effects.

3. Develop a knowledge base for the estrogenic action of xenobotics during development.

The availability of natural and synthetic estrogens, as well as antiestrogens (each with different pharmacological and toxicological properties), provides opportunities for development of methods and mechanistic approaches to predict risk. Estrogens are etiological agents in female reproductive tract toxicity, a major human health problem. Exposure to FDA-regulated estrogens and antiestrogens occurs in tens of millions of women. There is oral contraceptive exposure in over 100,000 pregnancies each year. In the U.S., about 5% of women will receive tamoxifen sometime during their lifetime. The fertility drug, clomiphene, is responsible for 1% of the live births. Phytoestrogen exposure of the human population via food is virtually universal; infants consuming soy formula are exposed to the highest doses. Estrogens are studied both with respect to their varying pharmacological and toxicological properties and their common mechanism of action. The division is constructing an estrogen knowledge base to predict hormonal activity of untested xenobiotics and to help generate hypotheses identifying gaps in regulatory data. These strategies are important in providing FDA with human and computational expertise and experimental flexibility in dealing with regulatory issues in these areas.

Hyperactivity, mental retardation and other neurological birth defects are another major public health concern. Since pregnant women are exposed to neuroactive xenobiotics, the FDA is responsible for assuring safety. Because functional brain damage can be difficult to detect anatomically (*e.g.*, mental retardation, schizophrenia, depression), functional testing is a necessity. Research is accordingly focused on the development of new strategies, concepts, and more sensitive and interpretable functional, anatomical, and neurochemical methods for detecting developmental neurological insult. This activity provides FDA with expertise regarding the use and interpretation of the newest techniques for assessing developmental neurotoxicity.

Women and their embryos/fetuses are exposed to a number of xenobiotics during pregnancy; most drugs are necessary to maintain maternal health and well-being. Mechanistic studies provide strategies and new concepts to help identify at-risk pregnancies as well as suggest possible intervention therapies (*e.g.*, the FDA issue of folate supplementation) that could circumvent developmental toxicity. Species and strain differences can be investigated *in vivo* (*e.g.*, Segment II developmental toxicity studies), in which maternal physiological factors can be monitored to determine any maternal effects of a chemical. Maternal plasma and embryonic/fetal drug levels can be measured to estimate embryonic exposure. Toxicity assessments in an *in vitro* whole embryo culture system allow for the evaluation of the effects of a chemical (or metabolite) in the absence of possible confounding maternal effects.

As part of NCTR's strategic move into the molecular biology of development, efforts toward identifying potential gene biomarkers critical to development are underway. One such effort involves insulin-like growth factors (IGFs), their binding proteins, and receptors. Since

diabetes increases the risk of birth defects even in women on insulin therapy, these molecular probes may be important in the etiology of such birth defects.

FY 96 Accomplishments and FY 97 Plans

Over the past 15 to 20 years, NCTR has been a leader in defining the normal and estrogen-altered reproductive tract developmental profile in the rat. This expertise provided the foundation for the reproductive and developmental toxicology involvement with the FDA Women's Health Issues initiatives. This same expertise and the well-defined estrogenic database created over the past 20 years has led to the initiation of a project to create and validate a computerized knowledge base utilizing experimental data to aid in the regulatory decision process, funded by a series of grants from FDA's Office of Women's Health. Additionally, scientists within the division are collaborators with Dr. Fred vom Saal (University of Missouri - Columbia) in a research project on endocrine disruptors funded by the NIEHS for four years.

Center studies have characterized the effects of two newer antiestrogens, droloxifene and toremifene, on developmental endpoints in the rat uterus. The results for toremifene are compared to those we previously described for the related drug tamoxifen in a submitted manuscript.

Work continues on the developmental effects of phytoestrogens. Alterations in reproductive tract morphology and biochemistry in rats treated neonatally with phytoestrogens at times soon after treatment were previously published. Results of follow-up experiments with sacrifices at six and ten months are being written for publication.

The Third International Phytoestrogen Conference, organized and sponsored by the DRDT and NCTR, was held in Little Rock, Arkansas, December 3-5, 1995. The papers from the symposium will be published in the Proceedings of the Society for Experimental Biology and Medicine.

Major studies, involving several NCTR research divisions, on several endocrine disruptors are in the planning stage. Estrogenic chemicals in foods, devices, drugs, veterinary medicines, and other FDA- regulated products are a developing concern. NCTR has taken a leadership role in the area, both within and outside FDA.

Assessment of the sensitivity and validity of behavioral and neuroanatomical measures of developmental brain damage continues. Scientists in this division have improved our ability to detect and assess minor functional abnormalities in rodents. Following a FY96 move into

a larger, consolidated laboratory, we now have one of the best equipped rodent behavioral laboratories in the U.S. This laboratory permits the in-depth assessment of as many as ten different behaviors in large numbers of rodents. We are especially well-equipped for detection of mild developmentally-induced hyperactivity and/or mental retardation in rodents. We routinely monitor 24-hour ambulatory and running wheel activity in as many as 16 pairs of animals simultaneously. We can assess short-term activity and response to stimulants in 16 to 24 rodents daily, providing the ability to sensitively detect even modest hyperactivity in large-scale rodent screening studies. Addition of the Morris water maze and expansion of the NCTR complex maze has substantially improved our ability to detect the equivalent of mild mental retardation in rodents.

FY96 saw an expansion of division research on the effects of prenatal retinoid exposure. It has shown that exposure in rodents at a period equivalent to the 4th to 6th week of human pregnancy causes abnormalities in brain development and function at doses far below those which cause morphological abnormalities. In FY96, the division utilized computerized image analysis to detect very specific retinoid effects on the pontine and olivary cerebellar relay nuclei during this sensitive period. It also initiated a major study (still on-going) of the neuroanatomical and developmental effects of retinoid exposure at three separate developmental stages. While still preliminary, this study suggests that major behavioral abnormalities occur with retinoid exposure well into the rodent equivalent of the 6th to 8th week of human pregnancy, again in the absence of major malformations. These findings suggest that the vitamin supplementation often begun at the time of pregnancy detection in humans may have the potential to create "silent" neurological effects, perhaps manifesting as mild hyperactivity. This disturbing possibility clearly merits further experimental scrutiny, and in FY97 the division will continue the above project while initiating a number of collaborative projects to more closely examine these effects. These collaborative efforts will include: 1) work with Dr. Jane Adams (University of Massachusetts) to attempt to determine whether cerebellar functional abnormalities seen in rodents might also occur in isotretinoin-exposed children; 2) work with Dr. Mark Stanton (EPA) to better characterize the functional cerebellar deficits in retinoid-exposed rodents; and 3) work with Dr. Frank Scalzo (UAMS/Arkansas Children's Hospital) to better characterize and understand feeding problems caused by retinoid exposure. This research will help to better clarify the neuroanatomical and functional effects of retinoid exposure in rodents and in humans.

Attention deficit disorder/hyperactivity still afflicts some 2% of children in the U.S. Over the past six years, division research has suggested that this syndrome is seen in rats after developmental exposure to a variety of compounds. Common to these syndromes is an unexpected 10% reduction in cerebellar weight. Neonatal exposure to antimitotics or dexamethasone, or prenatal exposure to low doses of retinoids, reduces adult cerebellar weight and is accompanied by behavioral hyperactivity. Consequently, they now believe that the cerebellum may be an especially sensitive target for developmental neurotoxicants. During FY97, the division with our collaborators at the University of Tennessee, are

examining such effects with state-of-the-art neuroanatomical and molecular biology techniques. To date, we have seen dexamethasone effects in all major regions of the cerebellum, as late as six months after a single dexamethasone injection on postnatal day seven have been seen. The division is currently assessing retinoid effects on survivors at this and earlier ages.

Microdialysis in various brain regions has become an established neurochemical assessment technique in our laboratory. In FY96, the Division of Reproductive and Developmental Toxicology (DRDT) initiated a project to assess the neurochemical response to FDA-regulated compounds such as fenfluramine in rodent neonates. The division staff also published a report on changes in the brain over the first 24 hours following microdialysis probe implantation. These findings have important implications for the interpretation of all results collected by this procedure. That work will continue in FY97 while also expanding the ability to use this technique to track cerebral metabolism of pharmaceuticals. This will be done in part by applying the technique of mass spectrometry to analyze methylphenidate (ritalin) levels in the brain and to trace the cerebral metabolism of this compound. It is also hoped that a better understanding of cerebral nitric oxide (NO) release with mass spectrometry will be achieved. If this application of mass spectrometry to cerebral microdialysis succeeds, the NCTR will have taken a major step forward in development of this important tool.

A classical Segment II teratology study of fumonisin B_1 (FB_1) in rabbits was completed. Decreased fetal body and organ weights were observed but only at the higher FB_1 doses. These doses also produced a significant amount of maternal toxicity, so it is unclear if the effects on fetal weight were secondary responses to maternal toxicity. The ratio of sphingonine to sphingosine, which was used as a biochemical marker of FB_1 exposure, was increased in a variety of maternal tissues but was not altered in fetal tissues. This suggests that FB_1 may not have crossed the placenta and further suggests that, in the absence of maternal toxicity, this compound does not appear to be a significant developmental toxicant.

The anticonvulsant drug, carbamazepine, is believed to cause neural tube defects in humans. The drug does produce developmental toxicity in animal models, and it is also capable of producing neural tube defects in a rodent whole embryo culture system in which rodent embryos are cultured directly in serum containing carbamazepine. Addition of rat or human hepatic metabolizing fractions increased the drug's embryotoxicity, somewhat suggesting that a metabolite might be the developmental toxicant. The tripeptide glutathione is able to detoxify a number of reactive metabolites. Experiments to manipulate embryonic concentrations of glutathione, either by decreasing its concentration by the action of the inhibitor buthionine sulfoximine or by increasing its concentration by addition of exogenous glutathione, have been inconclusive. During the next year, DRDT plans to develop methods to measure embryonic concentrations of glutathione to insure that the treatments are having

the desired effect. It also plans to test additional scavenging compounds to determine if they are able to decrease carbamazepine-induced embryotoxicity.

Another anticonvulsant drug, valproic acid (VPA), is known to produce neural tube defects in 1 to 2% of exposed human offspring as well as in animal models. The mechanism whereby VPA produces these defects is unknown. A number of anticonvulsants, including VPA, decrease the concentration of the vitamin folic acid. Evidence has indicated that this vitamin decreases the incidence of neural tube defects in humans. However, using a rodent whole embryo culture system, it has been demonstrated that neither folic acid nor a variety of other compounds involved in folic acid metabolic steps are able to decrease the frequency of neural tube defects produced by valproic acid. Preliminary evidence suggests that VPA might decrease the concentration of the primary donor of methyl groups for various methylation reactions, S-adenosylmethionine (SAM)(a product of one-carbon transfer reactions which utilize folic acid), and may decrease overall methylation of embryonic DNA. During the next year DRDT plans to further examine the effects of VPA on embryonic methylation reactions. And they plan to look at other compounds which produce neural tube defects to determine if alterations in SAM concentrations and methylation reactions might be a common mechanism leading to neural tube defects.

Based on its suspected morphoregulatory role in nervous system development, expression of the cell adhesion molecule (N-CAM) was examined in mouse embryos treated with a dose of VPA known to produce neural tube defects. Using a high sensitivity, high resolution Western blot system, N-CAM was detected in embryonic heads. No difference could be detected between VPA- and control-treated mice; however, localized differences in expression would not be detectable by this technique. Work has begun to use immunohistochemistry and *in situ* hybridization to determine if more localized alterations in expression could occur following treatment with VPA.

Additional mechanisms of developmental toxicity will be examined over the next year. These include alterations in stress protein synthesis as a mechanism for various developmental toxicants, including retinoic acid, heat and VPA.

A molecular biology capability has now been brought to this research area which enables developmental toxicity to be measured in terms of effects on fetal gene expression. This approach has been applied to determine the effects of maternal diabetes on fetal expression of eight insulin-like growth factors (IGFs) and binding protein mRNAs. The division has been able to identify a single binding protein, previously demonstrated to function as a growth inhibitor in other systems, that is upregulated in the growth retarded fetuses of diabetic dams and downregulated by insulin treatment. The extent of regulation of this binding protein gene is currently being quantitated by extremely precise molecular techniques.

The mechanism of retinoic acid-induced limb malformations will be analyzed at two different levels over the coming year using similar molecular techniques. First, the fetal distribution of nine distinct retinoic acid (RA) receptors and binding proteins will be determined to identify which are located in limb and other target tissues for RA-induced birth defects. This information could potentially allow the selection of retinoid derivatives which are more appropriate for therapeutic application due to a lack of binding to the "teratogenic" RA receptor or binding protein. Second, the effects of RA exposure on production of IGFs and IGF binding proteins in fetal limbs will be determined in order to identify which might be involved in RA-induced limb growth defects.

Investigations utilizing a laser scanning confocal microscope coupled with a Silicone Graphics Workstation have electronically imaged mouse embryos. The 3-D reconstruction of gestational day 9 to 11 embryos has provided insight into logistical problems of gestational age identification markers, processing shrinkage of tissues and organs, and storage, identification, and retrieval of the vast amounts of electronic data being generated. This next year will continue this effort to collect developmental data on normal embryonic and fetal development in the mouse and rat.

Significance to the FDA

Reproductive and developmental toxicology is investigating the effects of drugs and other xenobiotics regulated by FDA which collectively have extensive human exposure during pregnancy. By improving methods to detect and characterize developmental toxicants as well as determining the mechanisms for their effects, the FDA will be in a better position to predict the human developmental toxicity of regulated products and to advise regulated industry of appropriate procedures. This research area utilizes an integrated research approach by emphasizing molecular, endocrine, behavioral, limb and whole embryo *in vitro*, and pharmacokinetic techniques.

This combination of techniques and expertise is unequalled by any other single group in the FDA for studies in developmental toxicity and positions the individual scientist to be able to best contribute to the FDA regulatory arena.

RESEARCH PROJECTS

RESEARCH PROJECTS

This section contains a listing of the NCTR research projects. Following is an explanation of each header.

Project Number is a unique identifying number assigned to the NCTR projects. If the project has been changed, the identifying addendum number is located directly below the base number. The “E” number indicates a research project; the “P” number inidicates a preliminary experiment; and the “S” number indicates a research support project.

Principal/Co-Principal **Investigator**. The Principal Investigator (PI) is identified in bold type for each project. If the PI is not an NCTR employee, the PI's affiliation is printed as a footnote. For a complete listing of principal and co-principal investigators, see Index, "Principal Investigator by Project."

Status/**Res. Area**/GOAL. Status indicates if the project was "Active" or "In Review" at the end of calendar year 96, "Completed" during FY96 or first quarter FY97 or "Proposed" for FY97. The Res. Area (research area) indicates the abbreviation for the NCTR research area responsible for the conduct of the project. A listing of the full names of the research areas and its abbreviation is listed in the Table of Contents. The abbreviation of the related NCTR strategic goal is listed in all capitals. A description of these goals can be found in the Preface on page iii.

Title. In parenthesis following the "Title" is the acronym for the collaborating FDA Center, for those projects that are collaborative. Below is a listing of the full names of the centers.

Objective. A brief description of the purpose of the project. To locate a specific chemical related to a project, refer to the Index, "Projects by Chemical"

The FDA Centers

Center for Biological Evaluation and Research (CBER)
Center for Devices and Radiological Health (CDRH)
Center for Drug Evaluation and Research (CDER)
Center for Food Safety and Applied Nutrition (CFSAN)
Center for Veterinary Medicine (CVM)
Office of Regulatory Affairs (ORA)
Office of Women’s Health (OWH)

RESEARCH PROJECTS

Project Number	Principal/ Co-Principal Investigator(s)	Status/ Res. Area/ GOAL	Title	Objective
E0026200	**Cerniglia**	Active/ **Micro/** METH	Microbiological Diagnostic Methods: Development, Testing, & Evaluation	To improve diagnostic and epidemiological capabilities in bacteriology, parasitology, mycology, virology and serology as applicable to NCTR programs and projects.
E0260401 E0260412	**Chou** Fu	Active/ **Bio Tox/** CNPT	Effect of Caloric Restriction on DNA Binding and DNA Adduct Removal *In Vivo*	Determine whether or not caloric restriction (CR) does 1. affect the quantity of the total DNA adducts in livers from the mice treated with various carcinogens, namely aflatoxin B1 (AFB1), benzo(a)pyrene (BaP), and 4-aminoazobenzene (4-AAB) or its methylated derivatives, and skin cells from the mice treated with BaP; 2. alter the formation of the specific DNA adducts which may be responsible for the tumorigenicity of the chemical carcinogens; 3. modify the efficiency of removal of the specific DNA adducts, either enzymatically or non-enzymatically; 4. change activities of mouse-liver zenobiotic metabolizing enzymes, especially the hepatic glutatione S-transferase (GST) from the mice treated with AFB1 by measuring the *in vitro* and *in vivo* formation of AFB1-glutathione (GSH) conjugates.
E0628900	**Beland** Fullerton Melchior	Completed/ **Bio Tox/** CNPT	Determination of Chemically Induced Changes in DNA Structure and Sequence	To calculate the preferred conformations of specific DNA adducts and relate them to the mutagenic potential of the adducts, and, additionally, to determine the precise DNA sequence alterations formed during the replication of these adducts.

Project Number	Principal/ Co-Principal Investigator(s)	Status/ Res. Area/ GOAL	Title	Objective
E0637100 E0637102	**Leakey** Harmon	Active/ **Cal Res/** CNPT	Profiles-Hepatic Detoxicating Enzymes	1. To complete the collection and analysis of human post mortem liver. 2. Study development of human and animal drug metabolizing enzyme proteins by using immunoblotting techniques.
E0657300 E0657301 E0657302 E0657303 E0657304 E0657305 E0657306 E0657307 E0657308 E0657312 E0657318	**Fu** Dooley Herreno-Saenz Kadlubar Von Tungeln	Active/ **Bio Tox/** PRED	Tumorigenicity of Nitro-Polycyclic Aromatic Hydrocarbons (Nitro-PAHs) and their Metabolites in the Neonatal B6C3F1 Mouse	1. To determine the tumorigenicity in the neonatal B6C3F1 mouse of a series of parent nitro-PAHs, and their ring-oxidized and nitroreduced metabolites, that have been found to be mutagenic in the Salmonella typhimurium strains. 2. To determine structure-activity relationships of nitro-PAHs, as well as to determine the structural features that can affect tumorigenicity. 3. To determine if bacterial mutagenicity correlates with tumorigenicity of the nitro-PAHs selected for study. 4. To compare and assess the importance of ring-oxidation pathways and nitroreduction pathways for the metabolic activation of nitro-PAHs in relation to tumorigenicity.
E0657500 E0657501	**Lewis**	Active/ **Cal Res/** METH	Nutrient Digestibility and Nitrogen Balance Among Fischer 344 Rats and B6C3F1 Mice Fed NIH-31 Standard and Fortified Diets	Determine the digestibility and utilization of various dietary nutrients in the NIH 31 study and fortified diets by F344 rats and B6C3F1 mice.
E0660900	**Hart** Duffy Feuers	Completed/ **Cal Res/** CNPT	Role of Body Weight Changes on Mechanisms Modulating Chemical Toxicity in B6C3F1 Mice	Provide baseline data on effects of caloric restriction on alterations in physiological, metabolic, biochemical and molecular parameters.
E0662700	**Shaddock** Arlotto Casciano Schol	Active/ **Gen Tox/** METH	Reliable Methodology for Cryopreservation	1. To develop a reliable methodology for cryopreservation of isolated hepatocytes. 2. To assess the effects of cryopreservation on hepatocyte cultures with studies designed to measure changes in morphology, viability, recovery, metabolism and ability to repair DNA after chemical treatment.

Project Number	Principal/ Co-Principal Investigator(s)	Status/ Res. Area/ GOAL	Title	Objective
E0663811 E0663813 E0663814 E0663815 E0663816	**Ferguson** Holson Ali Hansen LaBorde Webb Paule	Active/ **R&D Tox/** CNPT	The Effect of Neonatal Dexamethasone (DEX) Exposure on Brain and Behavior in the Rat	1. To determine whether neonatal exposure to DEX reduces neonatal regional brain growth. 2. To determine whether neonatal DEX effects on brain growth resemble those caused by the antimitotic compound methylazoxymethanol (MAM). 3. To determine whether neonatal effects on brain growth persist into early adulthood. 4. If DEX effects are seen on neonatal brain growth, to determine whether these effects are reflected in behavior, and whether such behavioral effects resemble those caused by neonatal MAM exposure.
E0665000	**Poirier** Lyn-Cook	Active/ **Mol Epi/** CNPT	Altered Expression of Oncogenes in Rats Fed a Methyl-Deficient Diet	Determine expression following hypomethylation, of oncogenes either as altered mRNA or altered protein.
E0665300	**Lay** Chiarelli Churchwell Kadlubar Lin	Completed/ **Chemistry/** METH	Development of Mass Spectral Methods for the Identification of Trace Levels of Unknown Carcinogen-Nucleoside Adducts	To develop fast atom bombardment (FAB) mass spectral methods for the analysis of carcinogen-DNA adducts that have sufficient sensitivity for applications in human dosimetry.
E0665800 E0665801	**Poirier** Hass Lyn-Cook	Active/ **Mol Epi/** METH	Validation of *In Vitro* and *In Vivo* Transformation Assays Using Liver Cell Lines Previously Treated with Hypomethylating Carcinogens	Validate NCTR transformation responses (growth in soft agar and tumor induction) in aged, cryopreserved liver cell links treated with hypomethylating agents.
E0665900 E0665901 E0665921	**Lu** Hart Turturro	Active/ **Cal Res/** CNPT	Effects of Dietary Restriction on Cell Cycle Analysis in Rats and Mice	Study and compare the difference in cell proliferation between non-carcinogen and carcinogen treated animals, both ad libitum and CR restricted groups.
E0666900	**Fu** Von Tungeln	Active/ **Bio Tox/** CNPT	Comparative Regioselective and Stereoselective Metabolism of 7-Chlorobenz[a]anthracene and 7-Bromobenz[a]anthracene by Mouse and Liver Microsomes	To study the effects of chloro and bromo substituents on the regio- and stereo-selective metabolism of benz[a]anthracene by mouse and rat liver.

Project Number	Principal/ Co-Principal Investigator(s)	Status/ Res. Area/ GOAL	Title	Objective
E0667000 E0667001 E0667002	**Beland** Fullerton Heflich	Completed/ **Bio Tox/** CNPT	Relationship Between Administered Dose, DNA Adduct and Mutation Induction *In Vivo*	1. To determine the relationship between administered dose of 1,6-dinitropyrene and DNA adduct concentration in: a) target tissues for 1,6-dinitropyrene-induced tumors. This will test the hypothesis if DNA adduct concentrations in a target tissue are predictive of the tumor incidence, b) lymphocytes. This will test the hypothesis that DNA adduct concentrations in a readily-obtainable surrogate tissue (e.g., peripheral blood lymphocytes) are predictive of the tumor incidence in a target tissue (e.g., lung). 2. To determine the relationship between the administered dose of 1,6-dinitropyrene and the number and types of mutations induced in lymphocytes. This will test the hypothesis that the number and types of mutations in readily-obtainable surrogate tissue (e.g., peripheral blood lymphocytes) are predictive of the tumor incidence in a target tissue (e.g., lung).
E0667700 E0667701	**Poirier** Lyn-Cook Poirier Zapisek	Active/ **Mol Epi/** CNPT	A Study to Determine If the Carcinogenic Effect of a "Methyl-Deficient" Diet on Rats Can Be Reversed by a "MethylSufficient" Diet	Study methylation pattern of liver DNA and liver carcinogenesis in rats on methyl deficient diet then switched to methyl sufficient diet.
E0667900	**Evans** Cerniglia Fu	Completed/ **Bio Tox/** AGNT	Multinuclear NMR Studies on the Structure and Conformation of Metabolites and Adducts of Polycyclic Aromatic Hydrocarbons	To develop and improve onedimensional and two-dimensional NMR spectroscopy methods for structure elucidation of metabolites and DNA adducts of polycylic aromatic hydrocarbons and related compounds.
E0671000	**Fu** Kadlubar Von Tungeln	Completed/ **Bio Tox/** CNPT	Effect of Caloric Restriction on Metabolism and DNA Binding of Nitro-Polycyclic Aromatic Hydrocarbons (PAHs)	Determine effects of CR on patterns of oxidative and reductive metabolism, DNA adduct formation and distribution of nitro (PAHs)

Project Number	Principal/ Co-Principal Investigator(s)	Status/ Res. Area/ GOAL	Title	Objective
E0671700	**Pipkin** Hinson Lyn-Cook	Active/ **Gen Tox/** CNPT	The Stress Mobility Group of Proteins as Potential Biomarkers of Caloric Restriction In Aging Rats	Determine differences in High Mobility Group (HMG) proteins in young rats (ad lib); aged rats (ad lib) and aged rats (CR).
E0671900 E0671911 E0671921	**Ali** Bowyer Carrington Holson Lipe Melethil Newport Scallet Siitonen Slikker Sobotka Soliman	Active/ **Neuro Tox/** AGNT	Neurotoxicity Assessment of Prenatal, Postnatal, and Adult Exposure to Manganese in the Rat (CFSAN)	1. To determine whether administration of Mn during prenatal, postnatal and adult periods produces: i. any significant accumulation of Mn in plasma and different regions of the rat brain, ii. alterations in the dopaminergic neurotransmitter system, as evidence by a) changes in concentration of dopamine and its metabolites; b) in dopamine receptor binding and dopamine release, and c) in the rate limiting enzyme tyrosine hydroxylase. 2. To determine whether accumulation of Mn in the divalent (Mn=2) or trivalent (Mn+3) state is associated with producing neurotoxicity. 3. To determine if Mn accumulation and neurotoxicity is enhanced if administered to iron deficient rats during prenatal, postnatal or adult periods.
E0672900	**Thompson** Caper Siitonen	Active/ **Chemistry/** METH	Development of Method(s) for Analysis of Cadmium and Lead in Calcium Supplements by Graphite Furnace Atomic Absorption Spectrophotometry (CFSAN) (ORA)	1. Develop and validate a furnace AAS method for analysis of Cd and Pb in Ca supplements. 2. Analyze a sufficient number of different Ca supplements obtained from local health food businesses to determine applicability of method to different Ca matrices.
E0673000 E0673002 E0673003 E0673004 E0673005 E0673061	**Ferguson** Fogle Forrester Paule	Completed/ **R&D Tox/** PRED	Use of the Rodent Operant Test Battery (OTB) To Assess Neurobehavioral Toxicity: Preliminary Studies	To determine the most appropriate parameters and training/shaping methods with which to assess operant behavior in rodents using tasks which are currently used in the NCTR monkey OTB.
E0673200	**Lyn-Cook** Hass	Completed/ **Mol Epi/** CNPT	Characterization and Expression of p53 Proto-Oncogene in Dietary Restricted Rats: Biomarker for Susceptibility to Carcinogenesis	Examine the feasibility of p53 suppressor gene as a biological marker in examining the beneficial aspects of CR.

Project Number	Principal/ Co-Principal Investigator(s)	Status/ Res. Area/ GOAL	Title	Objective
E0673700 E0673701	**Beland** Marques	Completed/ **Bio Tox/** PRED	O-Acetylation of Carcinogenic N-Hydroxyarylamines	To synthesize a series of N-hydroxy derivatives from aromatic amine associated with cigarette smoke (aniline, o-, m-, and p-toluidine, 2,3-, 2,4-, and 2,6-dimethylaniline, 3- and 4-methoxyaniline, 2-naphthylamine, and 3- and 4-aminobiphenyl) and examine their ability to undergo O-acetylation both as a function of the acetyl donor and of the aromatic amine.
E0674100	**Morris** Domon McGarrity	Completed/ **Gen Tox/** CNPT	Biological Response Measurements in the Human	1. To develop methods whereby biomarkers of interest can be measured in the human lymphoblastoid cell line, TK+/-cell line. 2. To validate these methods by exposure of TK+/- cells to selected chemicals and quantification of the biomarkers of interest.
E0675100	**Holder** Joe Kendall Thompson	Completed/ **Chemistry/** AGNT	Investigation of Eight Mutagenic Heterocyclic Amines from *In Vitro* Studies Using Thermospray Mass Spectrometry (TSMS) (CFSAN) (ORA)	The application of an HPLC method previously developed in our laboratory for the quantification of eight mutagenic heterocyclic aromatic amines in various cooked meat samples (at the ppb level) using TSMS.
E0676000 E0676011	**Beland** Fullerton Olivero Poirier	Completed/ **Bio Tox/** AGNT	Incorporation of 3'-Azido-3'deoxythymidine into DNA of Target Tissues (CDER)	To determine if 3'-azido3'-deoxythymidine (AZT) becomes incorporated into the DNA of mouse vagina, a target tissue for tumor induction by AZT. To determine if AZT becomes incorporated into bone marrow cells, and if this is correlated with the reported bone marrow suppression and anemia associated with administration of this drug.

Project Number	Principal/ Co-Principal Investigator(s)	Status/ Res. Area/ GOAL	Title	Objective
E0676100 E0676111	**Slikker** Ali Appel Broening Newport	Completed/ **Neuro Tox/** CNPT	Postnatal Ontogeny of Neurotoxicity Susceptibility to Phenylisopropylamine Serotonergic Neurotoxicants (CDER)	1. To investigate the postnatal development of susceptibility to neurotoxic insult by using MDMA as a prototypic serotonergic neurotoxicant of the phenylisopropylamine class. 2. To correlate specific aspects of postnatal metabolic development derived from the literature to the ontogeny of neurotoxic susceptibility to phenylisopropylamine serotongergic neurotoxicants.. 3. To pharmacologically perturb the development of the dopaminergic neurotransmitter system in order to further clarify its role in events that surround the expression of susceptibility to phenyl neurotoxicants 4. To investigate the neurotoxic effects of fenfluramine on postnatal CNS development in the rat after optimization of postnatal dosing ages and post-exposure sacrifice time-points from studies w/MDMA.
E0676600	**Leakey** Casciano Domon Frame Harmon Lee McGarrity Morris Shaddock Zielinski	Active/ **Cal Res/** AGNT	*In Vitro* Metabolism of Topical Antimicrobials (CDER)	1. To determine the *in vitro* metabolic profiles of the topical antimicrobial, chlorhexidine digluconate (CHD) and p-chloro-m-xylenol (PCMX), in rat, human, and rhesus monkey liver preparations and in human lymphoblastoid cells transfected with individual drug metabolizing isozymes. 2. To assess the potential mutagenicity of the major metabolites of CHD and PCMX in human lymphoblastoid AHH-1 TK+/- cells, transfected with individual human cytochrome p450 isozymes. 3. To assess the validity of *in vitro* enzyme assays for predicting the metabolic fate of chemicals *in vivo*.
E0676700 E0676711	**Aidoo** Casciano Lyn-Cook	Active/ **Gen Tox/** PRED	Development of an Assay to Measure 6-Thioguanine-Resistant Rat T-Lymphocytes Treated with Mutagenic Agents *in vitro* (CDER) (CBER)	To develop techniques for *in vitro* mutagenicity studies with rat lymphocytes.

Project Number	Principal/ Co-Principal Investigator(s)	Status/ Res. Area/ GOAL	Title	Objective
E0676800	**Sheehan** Branham Hendry	Completed/ **R&D Tox/** KNLG	Estrogen-Induced Normal and Dysplastic Uterine Growth (CDER)	1. Evaluate a cross-transplantation system where both control and DES-exposed uterine tissues (neonatal donors) are introduced into the contralateral cheek pouches of control or DES-treated animals (adult hosts) that are either left intact, estrogen deprived (ovariectomized) or estrogen replaced (ovariectomized and estrogen implanted). 2. Perform homotypic and heterotypic recombination of epithelial and stromal tissue from control and DES-exposed uteri and study their estrogen-driven morphogenesis: a) *in vitro* using media supplemented with uterine tissue extracts and/or serum from the various host groups (control or DES-treated; intact, estrogen-deprived or estrogen--replaced); b) *in vivo* (within the cheek pouch) of various host groups (control or DES-treated; intact, estrogen-deprived or estrogen-replaced. 3. Examine and compare the influence of estrogen on c-myc and c-fos gene expression in the uteri of control vs. DES-treated hamsters.
E0676900	**Lu** Hart Zhang	Active/ **Cal Res/** CNPT	DNA Repair and Cellular Responses of Dietary Restricted Rats Exposed to Carcinogens	To examine DNA repair in glandular stomach & brain tissues of ad libitum fed male rats exposed to potent carcinogen MNNG or MNU, respectively. To develop and establish bromodeoxyuridine incorporation to study cell proliferation response in glandular stomach tissue of dietary restricted rats.
E0677000	**West** Hinson Lyle Rowland Swicord	Active/ **Bio Tox/** AGNT	Investigation of Neoplastic Transformation Induced *In Vitro* by 60 Hz Magnetic Fields (CDRH)	Determine if 60 Hz sinusoidally varying continuous wave magnetic fields can induce or promote neoplastic transformation *in vitro*.

Project Number	Principal/ Co-Principal Investigator(s)	Status/ Res. Area/ GOAL	Title	Objective
E0677100	**Thompson** Eppley Holcomb	Completed/ **Chemistry/** METH	Development of Method(s) for Analysis of Fumonisin in Corn Based Food/Feeds by HPLC and Fluorescence Detection (CFSAN)	1. Develop and validate an HPLC method for analysis of fumonisins in food/feeds using derivatization reagents that result in stable and sensitive fluorescent compounds. 2. Compare the method capabilities with the method currently being used at CFSAN. 3. Apply method in small surveys of corn-based foods.
E0677300	**Thompson** Harris Clower	Completed/ **Chemistry/** METH	Single Residue Method Evaluation for Fenbutatin Oxide in Apples, Oranges, and Cucumbers (CFSAN)	1. To develop and evaluate a single residue method for identification of the pesticide, fenbutatin oxide, in vegetables and fruits. 2. To make a comparison of the method developed for fenbutatin oxide with the existing standard method for analysis of this pesticide in vegetables and fruit.
E0677400	**Thompson** Billedeau Havery	Completed/ **Chemistry/** METH	Development of Methods for Analysis and Confirmation of Non-Volatile Nitrosamines and other N-Nitroso Compounds in Cosmetics and their Raw Materials (CFSAN)	1. To develop new extraction and cleanup methods for analysis of non-volatile nitrosamines (NVNAs) in a wide range of cosmetics and their raw materials using reversed-phase/high performance liquid chromatography (RP/HPLC) with chemiluminescence detection. 2. To identify and analyze NVNAs and other N-nitroso compounds in those cosmetics which result in longterm skin exposure.
E0677500	**Delclos** Blaydes Heflich Jacobson Smith	Active/ **Bio Tox/** AGNT	DNA Damage in Mammary Tissue, Liver and Nucleated Blood Cells of F344 Rats with Polyester Polyurethane (Microthane Foam) Implants (CDRH)	To develop methods for the detection of low levels of DNA damage produced by metabolites of 2,4- and 2,6-toluenediamine.

Project Number	Principal/ Co-Principal Investigator(s)	Status/ Res. Area/ GOAL	Title	Objective
E0678100	**Casciano** Domon McGarrity Morris Sotomayer	Active/ **Gen Tox/** PRED	Metabolism and Induction of Mutations by the Fermented Food-Borne Carcinogen Urethane in the Transgenic Human Lympho-blastoid Cell H2D6 (CFSAN)	To isolate and quantify spontaneous and urethane-induced mutations at the hypoxanthine guanine phosphoribosyl transferase (hprt) and the thymidine kinase (tk) loci in transgenic human lympho-blastoid cells expressing CYP2E1; to determine if urethane induces primarily single gene mutations or multilocus mutations; and to describe conditions which modulate metabolic activation of urethane to genotoxic metabolites.
E0678500	**Lay** Chiarelli Gay Sphon	Active/ **Chemistry/** METH	Development of FAB/MS and FAB/MS/MS Methodologies for the Analysis of Peptides (CFSAN)	To develop, using the combined resources available at CFSAN and NCTR, a capability for the analysis of peptides up to 3500 daltons via FAB/MS and FAB/MS/MS.
E0678600	**Cerniglia** Wang	Active/ **Micro/** PRED	Microbial Studies on Macronutrient Food Substitutes Phase I Validation Studies (CFSAN)	1. To validate the semicontinuous culture system for further studies on effect of macronutrient food substituents on the microbial activity and ecology of human intestinal micro-flora.
E0678700	**Shuttleworth** Cerniglia	Completed/ **Micro/** PRED	Degradation of Polycyclic Aromatic Hydrocarbon by Bacteria in Continuous Culture	1. To compare phenanthrene degradation kinetics among diverse strains of bacteria. 2. To compare the ability of the phenanthrene degrading bacteria to compete under various environmental conditions.
E0678800 E0678801 E0678820	**Berg**[**] **Feuers** Duffy	Active/ **Gen Tox, Cal Res/** AGNT	Acute Toxicity of Ganciclovir: Circadian Response and Effect of Dietary Restriction (CFSAN) (CDER) (CBER)	1. Determine if a circadian effect on the acute toxicity of ganciclovir can be demonstrated. 2. Determine if CR has an impact on the chrontoxicology of ganciclovir.

[**]University of Arkansas for Medical Sciences

Project Number	Principal/ Co-Principal Investigator(s)	Status/ Res. Area/ GOAL	Title	Objective
E0679100 E0679101	**West** Beer Rowland	Completed/ **Bio Tox/** AGNT	Neoplastic Transformation of Rhek-1 Human Epithelial Keratinocytes by UVA Radiation From the UVA Sun 3000s Lamp (CDRH)	Characterize the effects of UVA radiation on neoplastic transformation.
E0679500	**Casciano** Bradlaw Domon McGarrity Morris Page Shaddock	Active/ **Gen Tox/** PRED	Evaluation of Cytotoxic Properties of Chemical Components Associated with L-Tryptophan Contamination Using Transgenic Human Lymphoblastoid Cell Lines (CFSAN)	To identify biologically active contaminants of L-tryptophan through the use of cytotoxic indices in transgenic human lymphoblastoid cells.
E0679700	**Thompson** Syvertson Clower	Completed/ **Chemistry/** METH	Single Residue Method Evaluation for Azocyclotin in Citrus, Fruits and Vegetables (CFSAN)	To evaluate, adapt or develop a single residue method for analysis of azocyclotin residues in citrus, fruits, and vegetables for use by the FDA field laboratories.
E0679800	**Thompson** Syvertson Clower Eppley	Completed/ **Chemistry/** METH	Single Residue Method Evaluation for Dinoseb In Citrus, Fruits and Vegetables (CFSAN)	To evaluate, adapt, or develop a single residue method for analysis of dinoseb in citrus, fruits and vegetables for use by FDA field laboratories.
E0679900	**Thompson** Syvertson Clower	Completed/ **Chemistry/** METH	Single Residue Method Evaluation for Bitertanol Residues in Fruits and Vegetables (CFSAN)	To evaluate, adapt, or develop a single residue method for analysis of bitertanol residues in fruits and vegetables for use by FDA field laboratories.
E0680000 E0680011	**Sheldon** Warbritton	Completed/ **Cal Res/** CNPT	Development and Distribution of Optic Tract Lesions in DBA/2NNIA Mice, Restricted Versus Ad Libitum Diet	Determine development and distribution of degenerative lesions in optic tracts and nerves of DBA/2NNIA mice. Demonstrate delay in this is due to CR.
E0680500	**Casciano** Taylor Wamer	Active/ **Gen Tox/** CNPT	Does β-carotene Modulate the Effects of Carcinogens on Gene Expression (CFSAN)	Determine if β-carotene can modify the carcinogen induced expression of various oncogenes and tumor antigens in SV40 transformed CHO cells.

Project Number	Principal/ Co-Principal Investigator(s)	Status/ Res. Area/ GOAL	Title	Objective
E0681000	**Henderson** Cerniglia Johannessen	Active/ **Micro/** AGNT	The Role of Intestinal Microflora in the Metabolism of a Mammalian Metabolite of the Neurotoxin 1-methyl-4-phenyl-1,2,3,6-Tetra-hydropyridine (MPTP) (CFSAN)	To determine how anaerobic bacteria native to the human intestinal tract will metabolize 1-methyl-4-phenylpyridinium ion (MPP+), a mammalian metabolite of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) known to induce a condition similar to Parkinson's disease.
E0681300	**Shuttleworth** Cerniglia Davis Henderson	Active/ **Micro/** CNPT	Investigations of the Content and Fate of PAHs in Sediments Collected Near Commercial Fishing Areas and the Effects of Bioaugmentation with a PAH Degrading Mycobacterium Sp. in Environmental Microcosms	1. To determine the usefulness of a Mycobacterium sp. in the removal of organic pollutants from contam-inated sediments. 2. To test the ability of this Mycobacterium to mineralize PAHs in heavily contaminated sediments.
E0681600	**Aidoo** Lyn-Cook Wamer	Active/ **Gen Tox/** CNPT	Studies on Antioxidants: Evaluation of the Mutagenic Activity on N-Ethyl-N-nitrosourea (ENU) in the Rat (CFSAN)	1. To pre-treat F344 rats with anti-oxidant vitamins: β-carotene, L-as-corbic acid and dl-α-tocopherol in the drinking water for one week and then expose the animals to 100 mg/kg ENU (a direct acting muta-gen) or to simultaneously expose the animals to the antioxidants and 100 mg/kg ENU. 2. To determine the tissue concentrations of the vitamins from the liver and the spleen after exposure to use the spleen lymphocytes to measure the frequency of 6-thioguanine-resistant T-cells employing the rat lymphocyte clonal assay to evaluate the relationship between ENU-induced hprt locus mutations and antioxidants intake.
E0681800	**Scallet** Robl Sobotka	Completed/ **Neuro Tox/** AGNT	Characterization of the Neurobe-havioral Toxicity Associated with Low Dose Exposure to Domoic Acid in the Rat (CFSAN)(ORA)	To correlate a time and dose-re-sponse profile of the neurobe-havioral toxicity of single doses of domoic acid with associated neurohistopathological changes.

Project Number	Principal/ Co-Principal Investigator(s)	Status/ Res. Area/ GOAL	Title	Objective
E0682200 E0682211	**Paule** Ali Binienda Ferguson Gillam Johannessen Slikker Sobotka Taylor	Active/ **Neuro Tox/** AGNT	The Effects of Chronic MPTP Administration on Complex Brain Function, Neurochemistry and Neurohistology in the Rhesus Monkey (CFSAN)	Chronic administration of low doses of the dopaminergic neurotoxicant MPTP will lead to detectable alterations in 'cognitive' brain function in the absence of frank Parkinsonian symptoms.
E0682500	**Wolff** Dunkel Jackson Whittaker	Active/ **Bio Tox/** AGNT	Determination of Dose Levels to Be Used in Chronic Carcinogenicity Study of Iron Overload (CFSAN)	To determine the dose levels of carbonyl iron to be used in a subsequent chronic bioassay
E0682900	**Lipe** Ali Carrington Newport Slikker	Active/ **Neuro Tox/** AGNT	Effect of Manganese on the Concentration of Amino Acids in Various Regions of the Rat Brain (CFSAN)	1. To determine if exposure to manganese alters amino acid concentrations in selected regions of the adult rat brain. 2. To determine if exposure to manganese alters amino acid concentrations in selected regions of the developing rat brain.
E0683000	**Ali** Chen Feuers Newport Oriaku Slikker	Completed/ **Neuro Tox/** CNPT	Oxidative Stress-Induced Age-Related Neurotoxicity in Mice: Possible Mechanism of Action of METH and MPTP (CFSAN)	1. Determine whether neurotoxic doses of METH and MPTP produce alterations in protective enzymes known to decrease the effects of oxidative stress in mouse striatum. 2. Determine the effects of neurotoxic stress of METH and MPTP on dopamine and its metabolites in mouse striatum. 3. Determine whether any noted changes in protective enzymes occur before or after the depletion of dopamine in striatum. 4. Determine whether there are age-related changes in these protective enzymes and how these changes correlate with the depletion of striatal dopamine after METH or MPTP exposure.

Project Number	Principal/ Co-Principal Investigator(s)	Status/ Res. Area/ GOAL	Title	Objective
E0683400 E0683401 E0683402	**Feuers** Deluca York	Active/ **Gen Tox/** CNPT	The Effect of Age and Food Restriction on Glutathione S-Transferase Isozymes in C57BL/6N Mice	This study seeks correlations between aging, caloric restriction, and BHA administration with alterations in GSTase isozyme composition.
E0683500	**Hammons**	Completed/ **Mol Epi/** METH	Characterization of Cytochrome P-450IA2 through the Utilization of Photoaffinity Labeling	Develop a photoaffinity labelling method for characterizing the catalytic site of the cytochrome P-450 enzyme, P-450IA2.
E0683700	**Paule** Gillam Slikker	Active/ **Neuro Tox/** AGNT	Effects of Chronic Methylphenidate (Ritalin) Administration on 'Cognitive' Functions in the Rhesus Monkey (CDER)	To determine whether chronic treatment with relevant doses of the therapeutic agent methylphenidate (Ritalin) will result in detectable changes in specific 'cognitive' abilities in a nonhuman primate model of complex brain function.
E0683800	**Kodell**	Completed/ **Biometry/** METH	Adjusted P-values for Tests of Multiple Tumor Sites (CFSAN) (CDER)	To evaluate standard statistical procedures that are used for testing for the overall carcinogenic potential of a drug or chemical.
E0684000	**Colvert** Ferreira Holland Rafii	Active/ **Micro/** METH	Detection of Clostridium Botulinum Using Enzyme Linked Immunosorbent Assay and Polymerase Chain Reaction Techniques (ORA)	The primary objective is to develop better *in vitro* methods for the detection of C. botulinum.
E0684400	**Colvert** Holland Noah	Active/ **Micro/** METH	Production of Monoclonal Antibodies Against Vibrio Cholerae for Diagnostic Screening Tests: Establishment of Monoclonal Antibody Capabilities at NCTR (ORA)	A rapid method is needed to screen food samples for Vibrio cholerae at the FDA.
E0684500	**Paule** Clausing Appel	Active/ **Neuro Tox/** AGNT	Preliminary Assessment of a Method for Screening Potential Neurotoxic Effects of Prenatal Alcohol Exposure using Autoradiographic Measurement of Cellular Metabolic Markers (CDER)	1. To examine, validate and compare the utility of a number of biochemical markers of neurotoxic insult that can be detected using autoradiographic methods.

Project Number	Principal/ Co-Principal Investigator(s)	Status/ Res. Area/ GOAL	Title	Objective
E0684700	**Duffy** Aly Feuers Hart Pipkin	Completed/ **Cal Res/** CNPT	Effects of Chronic Caloric Restriction, Exercise and Isoproterenol on Cardiovascular And Metabolic Performance and Stress Proteins In Rats of Different Ages	1. Determine the effect of CR, physical fitness and cardiovascular states as a function of age; 2. assess the role of chronic CR in modulating the effect of chemicals that produce toxic effects on the cardiovascular system, and 3. determine the effects of CR, exercise, drugs and age on the expression of stress proteins in various tissues.
E0684800	**Littlefield** Hass Poirier	Active/ **Bio Tox/** AGNT	The Effect of Dietary Magnesium on the Induction of Tumors, Transformation of Cells, and Leukemia Incidence	1. To identify and evaluate the appearance of tumors, cell transformation, disruption of cell cycles and increased incidences of leukemia that may be related to dietary Mg deficiency, and 2. evaluate possible modulations of tumor expression through possible interactions of Mg and a carcinogenic, metal, such Ni.
E0685000 E0685011	**Aidoo** Heflich Manjanatha	Active/ **Gen Tox/** PRED	Lymphocyte Mutation as a Biomarker for Mammary Tumors Induced by 7,12-Dimethylbenz(a)-anthracene in Sprague Dawley Rats (CFSAN) (CDER) (CBER) (CDRH)	Determine if mutations at the hprt locus of lymphocytes from Sprague-Dawley rats treated with DMBA can be used as a biomarker for the induction of mammary tumors.
E0685200 E0685211	**Jackson** Sheldon Weis	Completed/ **R&D Tox/** AGNT	Doxylamine: Secondary Mechanisms of Carcinogenicity in Male B6C3F1 Mice (CDER)	To examine, histologically and biochemically, possible indirect mechanisms by which doxylamine, a nongenotoxic drug, produces hepatocellular ademonas and thyroid follicular cells ademonas in mice.
E0685300	**Yerokun** Heflich	Active/ **Gen Tox/** PRED	Construction of Transgenic Hamster Ovary Cells Expressing Arylsulfotransferases IV and Their Use in Studies of Molecular Mechanisms of Arylamine- and Polycyclic Aromatic Hydrocarbon-Induced Carcinogenesis	1. To construct a mammalian expression vector containing the AST IV gene and to transfect CHO cells with the recombinant vector, and 2. to use these transgenic cells in the hypoxanthine-guanine phosphoribosyl transferase (hprt) and adenosine phosphoribosyl transferase (aprt) mutation assays.

Project Number	Principal/ Co-Principal Investigator(s)	Status/ Res. Area/ GOAL	Title	Objective
E0685600 E0685611	**Valentine, C.** Valentine, J.	Completed/ **Gen Tox/** PRED	Metabolism of Carbamazepine by Human Lymphoblastoid Cells Expressing Human CYP450s	Specific human cytochromes P450s form the stable epoxide of CBZ and toxic metabolites. The epoxide, toxic metabolites, and atypical metabolites of CBZ found in the blood of patients taking this drug will correlate with metabolites formed by different human CYP450s.
E0685800	**Jackson** James	Active/ **R&D Tox/** CNPT	Requirements for Dietary Nucleotides and Folic Acid in Rapidly Dividing Cells: II. Effect of Partial Hepatectomy on Foci and Tumor Development	Determine if purified and semi-purified diets such as AIN-76A that lack preformed nucleotides will compromise DNA synthesis in tissues undergoing rapid cell proliferation and if this interference in DNA synthesis is associated w/alterations in deoxynucleotide pools & a greater risk for cell transformation.
E0685900 E0685911	**West** Beland Delclos Fu Rowland	Active/ **Bio Tox/** CNPT	The Transformation Potential of Nitro-Polycyclic Aromatic Hydrocarbons Assayed by the Rat Trachael Epithelial Cell (RTE) Systems	To optimize conditions for the transformation of RTE cells *in vitro* with nitro-PAHs. To characterize the metabolic capability of the primary RTE cell population using 1,6-dinitropyrene & 6-nitrochyrysene as substrates. To characterize the DNA-adduct profile in primary RTE cells after exposure to 1,6-dinitropyrene & 6-nitrochrysene. To determine the transformation potential of 1-nitrosopyrene, 2-nitropyrene, and 1-,2-, & 3-nitrochrysene, so as to extend the data base for evaluation of SARs w/nitro derivatives of pyrene & chrysene already begun under E-6742. To determine the transformation potential of certain nitro derivatives of benzo(a)pyrene and benzo(e)pyrene.

Project Number	Principal/ Co-Principal Investigator(s)	Status/ Res. Area/ GOAL	Title	Objective
E0686101 E0686111	**Littlefield** Poirier	Active/ **Bio Tox/** CNPT	Determination and Evaluation of Toxicity, Tumor-Promoting Activity, and Synergistic Interactions Between Granulocyte-Macrophage ColonyStimulating Factors and Agents (CBER)	To identify and quantitate the interactions between the cytokine granulocyte-macrophage colony-stimulating factor (GM-CSF) and chemotherapeutic agents in respect to toxicity, carcinogenicity, and/or the synergistic actions between CSFs and chemotherapeutic agents.
E0686501	**Hass** Chen Littlefield	Active/ **R&D Tox/** AGNT	Verification of Methapyrilene, a Human Antihistamine and a Rat Liver Carcinogen, as an Agent that Alters Phenotypic Expression of Transformed Cells	This study is the first phase of several possible sequential studies designed to investigate the potential uniqueness of MP as an anti-tumor agent. The objective of this study is to establish the hypothesis that MP at certain non-toxic concentrations is an anti-transformation agent.
E0686701 E0686711	**Hansen** Dial Grafton	Active/ **R&D Tox/** PRED	Investigations on the Mechanism of Valproic Acid-Induced Embryotoxicity *In Vitro* (CFSAN)(CDER)	To determine if 5-formyltetrahydrofolate, 5-methyltetrahydrofolate or folic acid is able to decrease the incidence of VPA-induced neural tube defects in rat embryos *in vitro*; To determine if L- or D-serine, formate, or MET is able to decrease the incidence of VPA-induced neural tube defects in rat embryos *in vitro*; To determine if pretreatment of rats with MET is able to decrease the incidence of VPA-induced neural tube defects in rat embryos *in vitro*.
E0686800	**Freni**	Active/ **Biometry/** AGNT	Fluoride in Public Drinking Water Systems and Human Fetal Health	Investigate the statistical association of exposure to fluoride in drinking water and fetal death, prematurity, low birth weight, and infant death in the counties and states in the U.S. included in project E-6733.

Project Number	Principal/ Co-Principal Investigator(s)	Status/ Res. Area/ GOAL	Title	Objective
E0686901 E0686911	**Binienda** Ali Ferguson Paule Scallet Slikker Taylor	Completed/ **Neuro Tox/** CNPT	The Effects of Prenatal Hypoxia on Postnatal Hippocampal Development in the Rat: Behavioral, Neurohistological and Neurochemical Studies	1. To assess complex brain function in the rat following prenatal hypoxia. 2. To correlate behavioral and neurohistological changes caused by prenatal hypoxia. 3. To evaluate the effects of hypoxia on monoamine systems in the brain using neurochemical methods.
E0687001	**Ahn** Kodell	Active/ **Biometry/** METH	Nonparametric Estimation and Testing of the Tumor Incidence Rate in Survival/Sacrifice Experiments (CFSAN) (CDER)	To develop a method to estimate the tumor incidence rate under the constraint that the tumor incidence rate is non-negative.
E0687101	**Freni** Ahn Eberhardt Hine Turturro	Active/ **Biometry/** AGNT	Caloric Intake and Human Health (The NHANES-1 Study) (CFSAN) (CDER)	Investigate whether caloric consumption is a predictor of human health in general, or of certain specific health effects.
E0687401	**Miller** Freeman Grahn Heinze	Active/ **Chemistry/** METH	Development of Devices/Methods for Determination of Food/ Seafood Quality (ORA) (CFSAN)	Assist FDA with problems incurred in testing seafood for decomposition by developing an expeditious assay for determining volatile and semivolatile organic compounds in spoiled seafood.
E0687501 E0687511 E0687521	**James-Gaylor** Poirier Wise	Active/ **Bio Tox/** CNPT	Mechanisms of Diet-Induced DNA Damage with Methyl Donor Deficiency (CFSAN)	Further the understanding of the mechanisms by which diet, as an environmental variable, can alter the susceptibility to cancer.
E0687701	**Wang** Campbell Cao Cerniglia	Active/ **Micro/** METH	Development of Species-Specific DNA Probes and PCR Methods for Rapid Detection of Anaerobic bacteria: Clostridium perfringens, C. clostridiiforme, C. leptum, Bacteroides distasonic, B. vulgatus, B. thetaiotaomicron ..	Develop simple, specific, sensitive and reliable methods for rapid identification and detection of anaerobic bacteria: Clostridium perfringens, C. leptum, C. clostridiiforme Bacteroides distasonis, B. vulgatus, B. thetaiotaomicron and other related species.

Project Number	Principal/ Co-Principal Investigator(s)	Status/ Res. Area/ GOAL	Title	Objective
E0687801 E0687811	**Lyn-Cook** Aidoo Casciano Taylor Wamer	Active/ **Gen Tox/** CNPT	Evaluation of the Effects of Dietary Antioxidants on Lymphocyte Function and Genotoxicity Induced in Young and Old Rats Exposed to DNA-damaging Agents *In Vivo* (CFSAN)	1. To determine the effects of the antioxidant vitamins on the genotoxicity induced by exposing mutagens/carcinogens to young and old rats. 2. To determine the effects of antioxidant vitamins on lymphocyte function in mutagen-exposed and non-exposed young and old rats.
E0687901 E0687912 E0687913 E0687914 E0687915 E0687916	**Fu** Casciano Contrera Kadlubar Teitel VonTungeln	Active/ **Gen Tox/** PRED	The Evaluation of Selected Benzodiazepine and Antihistamine Drugs in the Neonatal Mouse Tumorigenicity Bioassay and in Transgenic Human Lymphoblastoid Cells (CDER)	1. To determine if the neonatal mouse bioassay can be employed to evaluate the tumorigenic potential of therapeutic drugs. 2. To examine concurrently as positive controls the genotoxic carcinogens: 4-aminobiphenyl, benzo(a)pyrene, 6-nitrochrysene, & aflatoxin B1 3. To study the metabolism and DNA adduct formation of benzodiazepine and antihistamines drugs by mouse and human liver microsomes to determine which, if any, cytochrome P450 is responsible for metabolic activation in mice and humans. 4. Transgenic human lymphoblastoid cell lines expressing appropriate CYP isozymes will also be employed to study the mutations and DNA binding of the subject drugs.
E0688101 E0688111	**Hansen** Dial Grafton	Active/ **R&D Tox/** CNPT	Investigations on Carbamazepine (CBZ) Embryotoxicity *In Vitro* (CFSAN) (CDER)	1. To determine if exposure of embryos *in vitro* to carbamazepine (CBZ) alters normal growth and development. 2. To determine if a stable epoxide metabolite of CBZ or metabolic activation of CBZ by microsomes alters normal growth and development of embryos. 3. To determine if any of three folate derivatives will ameliorate potential embryotoxicity due to exposure *in vitro* to CBZ or its principle metabolite.

Project Number	Principal/ Co-Principal Investigator(s)	Status/ Res. Area/ GOAL	Title	Objective
E0688201 E0688211	**Wolff** Ali Contrera	Active/ **Bio Tox/** AGNT	Tumor Promotion and Neurochemical Changes in Mice During Chronic Feeding of the Antidepressant Fluoxetine (CDER)	1. To determine if chronic feeding of fluoxetine (Prozac) results in promotion of mouse mammary carcinomas. 2. To determine if chronic feeding of fluoxetine: a) produces changes in the concentrations of serotonin and its metabolite, 5-hydroxyindoleacetic acid, in different regions of the mouse brain; b) induces changes in serotonergic receptor and uptake sites in different regions of the mouse brain.
E0688301	**Evans** Deck Levine Luchtefeld	Completed/ **Bio Tox/** METH	NMR Spectroscopy of Sulfonylurea Herbicides (ORA)	1. To obtain H NMR spectra for analytical standards of 12 sulfonylurea herbicides. 2. To assign spectral peaks and confirm that proposed structures are correct. 3. To determine purities of the herbicide standards.
E0688401	**Slikker** Ali Holson Kwon Rottinhaus Sobotka	Completed/ **Neuro Tox/** AGNT	Developmental Neurotoxicological Assessment of Fumonisin (FB1) Toxicosis in Rats (CFSAN)	1. To assess complex brain function in the rat following prenatal exposure to FB1 2. To evaluate FB1 effects on monoamine systems in the brain using neurochemical methods. 3. To evaluate FB1 effects on membrane dysfunction by a determination of brain membrane fluidity. 4. To evaluate FB1 effects on astrocytes and on brain cell sphingolipid biosynthesis. 5. To correlate behavioral and neurochemical changes caused by prenatal FB1 toxicosis.
E0688501	**Branham** Andrews Burroughs Degeorge Fishman Medlock Sheehan Streck	Active/ **R&D Tox/** KNLG	Effects of Therapeutic Antiestrogens on Postnatal Uterine Development in the Rat (CDER)	To assess the developmental toxicity of the antiestrogens toremifene, droloxifene, and ICI 164,384 in the developing rat uterus as measured by uterine weight, luminal epithelium morphology and ultrastructure, and uterine gland genesis. To assess uterine estrogen receptor modulation by neonatal antiestrogen exposure.

Project Number	Principal/ Co-Principal Investigator(s)	Status/ Res. Area/ GOAL	Title	Objective
E0688601	**Fishman** Branham Sheehan Streck	Active/ **R&D Tox/** KNLG	In Situ Expression of Estrogen Receptor (ER) Protein and mRNA in the Developing Reproductive Tract (CDER)	To analyze estrogen effects on ER levels in the developing reproductive tract at the cellular and molecular genetic level.
E0688701 E0688711	**Ali** Cadet Freyaldenhoven Newport Slikker	Active/ **Neuro Tox/** CNPT	Evaluation of Constitutive and Stress-Induced Levels of Expression of Heat-Shock Proteins in Cu/Zn-Superoxide Dismutase Transgenic Mice	1. Determine whether there are significant differences in constitutive HSP expression in Cu/Zn-Superoxide Dismutase-transgenic mice versus non-transgenic littermate controls, C57BL/6N controls as well as CD1 controls. 2. Determine whether there are significant differences in the expression of inducible forms of HSPs after exposure to MPTP in SOD-transgenic mice versus non-transgenic littermate controls, C57BL/6N controls as well as CD1 controls. 3. Determine whether there are significant differences in the timeframe of the HSP response in SOD-transgenic mice versus non-transgenic litter-mate controls, C57BL/6N controls as well as CD1 controls. 4. Determine whether there exists differential expression of isoforms of HSP in SOD-transgenic mice versus non-transgenic mice littermate controls, C57BL/6N controls as well as CD1 controls. 5. Evaluate if induction of HSP correlates with the depletion of dopamine in SOD-transgenic mice versus non-transgenic controls, C57BL/6N controls as well as CD 1 controls.

Project Number	Principal/ Co-Principal Investigator(s)	Status/ Res. Area/ GOAL	Title	Objective
E0688801 E0688811	**Chou** Aidoo Allaben Bowers Casciano Gaylor Giri Green Hinton James Kodell Morris Roth Sahu Sotomayer Warbritton	Active/ **Bio Tox/** AGNT	A Collaborative Research Proposal to Assess Cancer Risk Posed by Intermittent Exposure to Aflatoxin B1 in Rats (CFSAN)	1. To test the hypothesis that a chemically induced tumor incidence is a function of the accumulated lifetime exposure, and is predictable from the average daily dose for various dosing regimens, such as continuous and intermittent dosing. 2. To study correlations between the chemically-induced tumor incidence and various biomarkers of the initiation and the promotion stage of carcinogenesis for continuous and intermittent dosing. 3. To determine whether nutritional status can alter the sensitivity to carcinogen dose, the expression of various biomarkers, and cancer risk assessment.
E0688901	**Shaddock** Feuers	Active/ **Gen Tox/** METH	Effect of Cryopreservation and Long-Term Storage of Primary Rodent Hepatocytes on I25 I-Insulin Uptake and Binding and the Regulation of Hepatic Pyruvate Kinase by Insulin and Glucagon Treatment	1. The primary objective of this study will be to measure Km and Vmax of hepatic pyruvate kinase for phosphoenol pyruvate in freshly isolated and cryopreserved rodent hepatocytes. 2. Evaluate the ability of insulin to dephosphorylate and glucagon to phosphorylate hepatic pyruvate kinase in freshly isolated and cryopreserved rodent hepatocytes. 3. Evaluate the ability of insulin to stimulate hepatic pyruvate kinase synthesis and glucagon to decrease the levels of pyruvate kinase synthesis in freshly isolated and cyropreserved rodent hepatocytes 4. Measure 125 I-Insulin uptake and binding in freshly isolated and cryopreserved rodent hepatocytes.

Project Number	Principal/ Co-Principal Investigator(s)	Status/ Res. Area/ GOAL	Title	Objective
E0689001 E0689011	**Streck** **Rajaratnam** Fishman Streck Webb	Active/ **R&D Tox/** CNPT	Effects of Maternal Diabetes and Insulin on Fetal Expression of Insulin-like Growth Factor and Insulin-like Growth Factor Binding Protein mRNAs (CDER)	To determine whether experimentally inducing diabetes in pregnant rats by treatment with streptozotocin will alter fetal expression of insulin-like growth factor mRNAs and insulin-like growth binding protein mRNAs. To determine to what extent restoring normoglycemia in pregnant diabetic rats by treatment with insulin will restore the normal pattern of fetal expression of insulin-like growth factor mRNAs and insulin-like growth factor binding protein mRNAs.
E0689101 E0689111	**Medlock** **Burroughs** Faber Hughes Sheehan Whitten	Active/ **R&D Tox/** KNLG	Alterations in Reproductive Tract Morphology and Biochemistry in Rats Treated Neonatally with Phytoestrogens (CFSAN)	1. To determine if the phytoestrogens, when given neonatally, alter estrogen receptor and progesterone receptor concentrations in the uterus and brain at 6 and 10 months in the same manner as DES. 2. To determine if the phytoestrogens, when given neonatally cause the same morphological alterations in the female reproductive tract at 6 and 10 months as DES. 3. To determine if the phytoestrogens, when given neonatally, elicit the same induction of the c-ras, c-myc and c-fos oncogenes as DES.
E0689201	**Mittelstaedt** Heflich Smith	Completed/ **Gen Tox/** METH	PCR-DGGE and PCR-SSCP Analysis of Mutation in the HPRT Gene of Rat Lymphocytes	Utilize the polymerase chain reaction (PCR) in conjunction with denaturing gradient gel electrophoresis (PCR-DGGE) and single-strand conformation polymorphism (PCR-SSCP) to detect and analyze hprt mutation in exons 3 and 8 from lymphocytes isolated from rats.

Project Number	Principal/ Co-Principal Investigator(s)	Status/ Res. Area/ GOAL	Title	Objective
E0689301	**Gaylor**	Completed/ **Biometry/** METH	Preliminary Estimate of Tumor Potency Based on the 90-Day Maximum Tolerated Dose (CFSAN) (CDER)	To examine the ratio between the maximum tolerated dose (MTD) based upon a 90-day study and the tumor potency for a large data-base, in order to obtain estimates of low-dose cancer risks from MTD, without conducting a two-year chronic study.
E0689401 E0689411 E0689421	**Teitel** Kadlubar Lin	Active/ **Mol Epi/** PRED	Chemoprotection of DNA Adducts of 2-Amino-1-methyl-6-phenylimidazo-[4,5-b]pyridine in the Rat	To examine the effect of the GSH S-transferase inducers, phenethyl-isothiocyanate, diallyl sulfide (DAS), 5-(2-pyrazinyl)4methyl1,2dithiol 3-thione (Oltipraz), garlic powder, cabbage powder, 2(3)-tert-butyl-4-hydroxyanisole (BHA), kahweol palmitate, cafestol palmitate, quercetin, tannic acid, a-angelical-actone, Green tea, and ethoxyquin on the metabolism and DNA adduct formation of the food-borne carcinogen, 2-amino-1-methyl-6-phenylimidazo[4,5-b]-pyridine, in the Fischer 344 rat.
E0689601	**Kodell** Ahn	Active/ **Biometry/** METH	Attribution of Tumor Lethality in the Absence of Cause-of-Death Information	To develop a nonparametric procedure for estimating distributions of time to onset of and time to death from occult tumors in the absence of cause-of-death information. To develop a method for imputing the number of fatal tumors in an experiment that lacks cause-of-death data, in order to modify the IARC cause-of-death test. To develop a procedure for estimating the lag time between onset of and death from an occult tumor, when cause-of-death data are unavailable. To illustrate the new procedures using data from the PCR studies.

Project Number	Principal/ Co-Principal Investigator(s)	Status/ Res. Area/ GOAL	Title	Objective
E0689701	**Hobbs**[***] Brand Griffin Kadlubar Schlenk	Completed/ **Tech Adv**[****]**/** AGNT	Accumulation of Copper in Edible Muscle of Channel Catfish (Ictalurus punctatus) Following Exposure to Water Borne Copper Sulfate (CVM)	To examine the effect of water of known chemical composition and constant temperature on the uptake, tissue distribution, and elimination of copper in fingerling channel catfish following exposure to copper sulfate at disease treatment levels.
E0689801 E0689813	**James-Gaylor** Basnakian Gaylor Hart Kammula Mishra Muschelishvili Pogribny Stratmeyer Turturro	Active/ **Bio Tox/** CNPT	Mechanisms of Foreign-Body Carcinogenesis: The Role of Inflammation, Proliferation, and Cell Death (CDRH) (CFSAN)	1. At the descriptive level: to determine with *in situ* immunohisto-chemical techniques, a) the early infiltration of CD4+, CD8+ T lymphocytes, B cells and inflammatory leukocytes into the area immediately adjacent to implant material... 2. At the mechanistic level: determine a) whether rates of proliferation and apoptotic cell death are altered in cells adjacent to implant material. 3. At the prognastic level: define the "survival index" in the early stages of foreign-body carcinogenic process that would be predictive of the subsequent development of dysplastic tissue and/or local sarcoma.
E0690001	**Khan** Cerniglia Eirkson Jones	Active/ **Micro/** METH	Development of a Detection Method for Tracking Genetically Engineered Microorganisms using Polymerase Chain Reaction and DNA-DNA Hybridization Methods (CVM)	To develop a rapid and sensitive detection method for tracking genetically engineered micro-organisms (GEMS) in environmental microcosms.
E0690101	**Pothuluri** Assaf Cerniglia Nawaz	Active/ **Micro/** METH	Microbial Degradation of Drugs and Feed Additives Used in Fish Farming (Aquaculture) (CVM)	To develop a standardized method to evaluate the biodegradation of drugs and feed additives used in fish farming (aquaculture). To determine the biodegradation rates and metabolic fate of the antibiotic erythromycin in aquaculture water and sediments.

[***]Oakridge Institute for Science and Education

[****]Technology Advancement

Project Number	Principal/ Co-Principal Investigator(s)	Status/ Res. Area/ GOAL	Title	Objective
E0690201	**Kodell** Chen Lin	Active/ **Biometry/** PRED	Bioassays of Shortened Duration for Drugs: Statistical Implications (CDER)	To conduct a Monte Carlo simulation study to evaluate the effect that terminating rodent bioassays at 18 months (or earlier) instead of 24 months would have on the statistical power to detect carcinogenic human drugs.
E0690301 E0690311	**Bowyer** Clausing Davies Gough Holson Newport Sandberg Slikker Stewart	Active/ **Neuro Tox/** CNPT	Factors Affecting the Neurotoxicity of Amphetamines and Related Compounds (CDER)	1. To determine how age, mode of administration, and environmental temperature during drug exposure alter the neurotoxicity of fenfluramine and methylphenidate. 2. Measure the effects of age and environmental temperature on the pharmacokinetics of several of the amphetamines. 3. The effect of neurotoxic doses of METH on the blood-brain barrier will be assessed to determine whether a "leaky" or damaged blood-brain barrier results from such exposure, and whether aging potentiates the likelihood of such damage. 4. The role of glia in METH and d-fenfluramine neurotoxicity will be assessed by elucidating the time-course of METH-induced gliosis, and by assessing the role of glia-derived neurotrophic growth factor (GDNF) in such neurotoxicity. 5. Neuroprotective compounds, neurotoxins, or compounds which affect energy utilization will be introduced into the striatum via microdialysis, while closely controlling body temperature, to determine if these compounds alter hyperthermia-induced METH neurotoxicity. 6. Ascertain whether the dopamine and serotonin depletions caused by continous nonacute exposure to low levels of METH via osmotic mini-pump are also dependent upon environmental and body temperature.

Project Number	Principal/ Co-Principal Investigator(s)	Status/ Res. Area/ GOAL	Title	Objective
E0690401 E0690411	**Ferguson** Holson	Active/ **R&D Tox/** PRED	Development of Techniques for Producing and Measuring Attentional Deficit Hyperactivity in Rats (CFSAN)(CDER)	1. Further development and characterization of existing techniques to detect behavioral hyperactivity. 2. Development of new behavioral techniques for assessing activity and attention in the rat. 3. Use of the above techniques to assess the impact of neonatal lead or dexamethasone exposure on activity and attention.
E0690501	**Holson** Ferguson Gough Hansen LaBorde Paule	Active/ **R&D Tox/** AGNT	Neural and Functional Teratogenesis of Retinoids in the Rat (CDER)	1. To determine age-specific retinoid dosage levels which produce no more than a 20% reduction in viability and do not increase the incidence of major morphological abnormalities. 2. To identify and characterize the age-specific functional and neurological alterations produced by the above doses. 3. To assess within-animal and within-litter correlations between functional and underlying neurological abnormalities induced by retinoids.
E0690601 E0690611	**Manjanatha** Aidoo Casciano Heflich Lyn-Cook Mittelstaedt Shelton	Active/ **Gen Tox/** PRED	Quantitative and Molecular Analysis of 7,12-Dimethylbenz[a]-anthracene-Induced Mutations in the Model Blue Rat: Comparison of Mutagenesis in the Transgene lacI with the Endogenous Gene hprt and Cancer Genes H-ras and P53 (CFSAN) (CDER) (CBER)	1. To determine the mutant frequency and mutation spectrum of the lacI transgene of the Blue Rat following exposure to DMBA in surrogate and target tissues and compare these mutant frequencies and mutational spectra to those determined in Objectives 2 and 3. 2. To determine the mutant frequency and mutation spectrum of the endogenous hprt reporter gene in T-lymphocytes from the spleens of Fisher 344 and Blue Rats following exposure to DMBA. 3. To induce mammary tumors in Fischer 344 rats and Blue Rats by exposure to DMBA and screen tumor DNA for mutations in the oncogene, H-ras and the tumor suppressor gene, p53.

Project Number	Principal/ Co-Principal Investigator(s)	Status/ Res. Area/ GOAL	Title	Objective
E0690801	**Zheng** Kodell	Active/ **Biometry/** METH	Properties of the Hazard and Survival Functions of the MVK Stochastic Carcinogenesis Model	Investigate mathematical properties of the MVK stochastic carcinogenesis model to deepen understanding and enlarge applicability of the MVK model. Study the two most important quantities of this model: the hazard and the survival function. Study the joint distributional properties of the numbers of initiated and malignant cells; Develop parameter estimation procedures so that the model can be fitted to real data; Exploit possible generalizations and extensions of this model.
E0690901	**Chen** Ahn Tsong	Active/ **Biometry/** METH	A Linear Mixed Effects Model for Analysis of Data for Stability Studies (CDER)	1. To investigate the methods proposed in the literature for improvement of the current FDA recommended procedure. 2. To investigate and develop a linear mixed effects model for statistical analysis of stability of a drug or biological product. 3. To develop a procedure for estimating a confidence limit on the predicted response for determining expiration period of a drug or biological product. 4. To investigate and develop computational procedures for estimating the regression coefficients of the fixed effects, mixed effects, and random effects, and variance components. 5. To investigate and develop procedures for testing the equality of variances of different batches. 6. To investigate and develop procedures for testing the equality of regression coefficients when the batch variances are unequal. 7. To compare the linear mixed effects approach with the current procedure recommended by the FDA.

Project Number	Principal/ Co-Principal Investigator(s)	Status/ Res. Area/ GOAL	Title	Objective
E0691001	**Gaylor** Chen	Active/ **Biometry/** PRED	Upper Limit for the Sum of the Risks of the Components in a Mixture and an Optimum Strategy for Risk Reduction	To develop a simple upper bound estimate of multiplicative risk factors and develop a simple upper bound estimate of the sum of the risks of components in a mixture. Utilize these upper limits to develop an optimum strategy for the expenditure of funds to reduce uncertainty in risk estimates.
E0691101	**Gaylor**	Completed/ **Biometry/** PRED	Correlation of Body Weight and Tumor Incidence in Female Mice	To determine if there is a difference in the body weight for female BALB/c mice that developed tumors compared to animals without tumors in the ED01 Study.
E0691201 E0691211 E0691221	**Wolff** Ali Whittaker	Active/ **Nutri Tox/** AGNT	Cellular and Molecular Responses to Chronic Iron Overload in Animal Models (CFSAN)	1. To determine the health effects of chronic iron overload in mice and rats. 2. To determine neurochemical changes after chronic iron overload in mice and rats. 3. To develop an animal model for identifying the cellular and molecular mechanisms underlying the hepatic and pancreatic effects of chronic iron overload which are characteristic of the human disease idiopathic hemochromatosis and possible neurochemical mechanisms which associate effects of iron with neurological disorders, e.g., Parkinson's and Alzheimer's diseases.

Project Number	Principal/ Co-Principal Investigator(s)	Status/ Res. Area/ GOAL	Title	Objective
E0691401 E0691411 E0691421	**Frederick** Fogle Paule	Active/ **Neuro Tox/** PRED	Validation of the NCTR Rodent Operant Test Battery as an Adjunct to the NCTR Primate Operant Test Battery: Implications for the Areas of Risk Assessment and Prediction of Neurobehavioral Toxicity	1. To determine the acute effects of a variety of prototypic psychotropic agents on rodent performance in an operant test battery (OTB) containing tasks designed to model several complex brain functions. 2. To determine the relative sensitivities of the behavioral endpoints monitored in the rodent OTB to pharmacological disruption. 3. To compare and contrast the acute effects of these psychotropic a gents on rodent and primate OTB performance to determine the degree to which behavioral findings in rodents can be extrapolated to primates. 4. To validate the use of rodent operant performance as useful predictors of neurobehavioral toxicity. 5. To add to existing knowledge of the neurochemical and neurophysiological basis of complex brain functions.
E0691501	**Hansen** Pauken Sonneborn Terry	Active/ **R&D Tox/** PRED	Stress Protein Expression Following Treatment with Developmental Toxicants *In Vitro* (CDRH)	To determine if mRNAs for stress proteins are synthesized by treatment with various developmental toxicants (valproic acid, lithium, ethanol, retinoic acid, and heat) in a rodent whole embryo culture system; to determine the kinetics of stress protein mRNA syntheses; to determine if this mRNA is translated into newly synthesized stress proteins; and to determine location of stress proteins in treated embryos by immunohistochemical detection.
E0691701	**Morris** Domon Richardson	Completed/ **Gen Tox/** AGNT	Cell Proliferation and Programmed Cell Death in Human Lymphoblastoid Cells (AHH-1 and TK6) Exposed to the Protein Cross-Linking Agents, Methyl Acetimidate and Dimethyl Adipimidate	1. To determine the effect of exposure to MAI and DMA on cell proliferation in human lymphoblastoid cells by flow cytometric analysis of BrdU/DNA. 2. To evaluate the cytotoxic response of human lymphoblastoid cells to MAI and DMA by a flow cytometric-cell viability assay.

Project Number	Principal/ Co-Principal Investigator(s)	Status/ Res. Area/ GOAL	Title	Objective
E0691801	**Terry** Hansen	Active/ **R&D Tox/** PRED	Immunohistochemical Localization of Folate in the Neural Tube at the Time of Closure (CFSAN)	To determine if folic acid and/or 5-methyltetrahydrofolate is present in the neural folds at the time of closure of the neural tube in untreated mouse and rat embryos; to determine if the location or quantity of folate present in the neural folds at the time of closure is altered by treatment with valproic acid which produces neural tube defects; and to determine if the location or quantity of folate present in the neural folds at the time of closure is altered by supplementation of the diet with folic acid.
E0691901	**Ahn** Barton Chen Hertzberg Kodell Springer	Active/ **Biometry/** METH	Simulation Study on Reducing Conservatism in Risk Estimation for Mixtures of Carcinogens (CFSAN)	To conduct a simulation study of methods for estimating upper bounds on excess cancer risk from exposure to a mixture of carcinogens, under the assumption of low-dose additivity of risks; to compare the common procedure of simply summing individual upper bounds on risk to less conservative estimating procedures, in order to investigate the reduction of conservatism in the total risk estimate; to determine if the reduction in conservatism achieved by the less conservative procedures makes it worthwhile for regulatory agencies to change from the common practice of summing upper bound risk estimates.

Project Number	Principal/ Co-Principal Investigator(s)	Status/ Res. Area/ GOAL	Title	Objective
E0692001	**Doerge** Divi	Active/ **Chemistry/** CNPT	Toxic Hazards from Anti-Thyroid Chemicals (CVM)	Determine inhibition mechanisms for environmental goitrogens using purified thyroid peroxidase and lactoperoxidase; Determine the mechanism for covalent binding suicide substrates to purified peroxidases using electrospray mass spectrometry to analyze intact adducted proteins and/or proteolytic fragments; Determine mechanism of goitrogen uptake into isolated thyroid cells in primary culture and subsequent inhibition of iodination/coupling reactions involved in thyroid hormone synthesis; Determine the structure-activity relationship for uptake of goitrogens into the thyroid and inhibition of thyroid hormone synthesis rats.
E0692201	**Sutherland** Cerniglia Eppley Freeman Wilkes	Active/ **Micro/** AGNT	Microbial Metabolism of Fumonisin (CFSAN)	The hypothesis of this project is that certain micro-organisms have the ability to metabolize toxic fumonisins to other compounds, which may correspond to unknown mammalian metabolites. The objective is to identify the major microbial metabolites of fumonisins for use in mammalian studies.
E0692301 E0692311 E0692321	**Binienda** Ali Flynn Kim Rountree Scallet Slikker	Active/ **Neuro Tox/** AGNT	3-Nitropropionic Acid (3-NPA) Hypoxia in them Rat: Neurochemical and Neurohistological Studies (CFSAN)	1. To evaluate the effects of the developmental neurotoxin 3-NPA on NMDA, dopaminergic and serotonergic systems using neurochemical methods. 2. To evaluate the neurohistological effects of calcium-mediated vs. serum -mediated stimuli on the expression of stress proteins (c-fos). 3. To correlate 3-NPA toxicity with age.

Project Number	Principal/ Co-Principal Investigator(s)	Status/ Res. Area/ GOAL	Title	Objective
E0692401 E0692411 E0692421	**Duffy** **Lu** Allaben Chanderbhan Feuers Hart Hass Hattan Leakey Lewis Lyn-Cook Pipkin Tang Taylor Turturro	In Review/ **Cal Res/** CNPT	Effect of Different Levels of Caloric Restriction on Physiological, Metabolic, Biochemical, Immunological, Molecular, and Body Composition Variables in Rats (CFSAN) (CDER)	To determine how various levels and durations of CR affect physiological function, enzymes related to intermediary and drug metabolism, hormonal regulation, blood chemistry, etc; Determine the relationship between BF, FFM, TBW, and TOBEC as a function of strain, age, mass, and nutritional status in rats; Validate and automate the use of a new noninvasive electromagnetic scanning device to measure BF, FFM, and TBW and to compare the results to a conventional chemical fat extraction technique; Determine if CR alters the relative quantity and disposition of various types of lipids such as cholesterol, phospholipids, free fatty acid, etc. in various tissues, as well as in urine, feces, and blood serum; Develop control data related to CR that can be used by CFSAN to evaluate the toxicity and efficacy of low calorie foods, food additives, and food substitutes; Determine temporal and environmental factors that modulate the toxicity of foods, food additives, and food substitutes; Develop experimental methods for utilizing CR in the chronic bioassay; Develop control data for a reference purified diet that has been formulated to conform to long-term nutrient requirements of rodent animal models typically utilized in toxicology and nutrition studies; Provide control data for the calculation of risk and levels of safety related to food and drug toxicity.

Project Number	Principal/ Co-Principal Investigator(s)	Status/ Res. Area/ GOAL	Title	Objective
E0692501	**Howard** Cashman Doerge	Active/ **Bio Tox/** CNPT	DNA Adduct Formation by Nicotine Metabolites	1. Determine the structural identity of the nicotine delta 1',2'- and delta 1',5'-iminium ion DNA adducts, and modify existing 32p-postlabeling techniques to detect the adduct. 2. Quantify the presence of these adducts *in vitro* and *in vivo* in mice.
E0692601	**Bowyer** Frame Lyn-Cook Slikker Stewart Tank	Active/ **Neuro Tox/** METH	Implementation of Molecular Biological Techniques for Assessing Changes in Neurogrowth/ Neurotrophic Factors after Exposures to Neurotoxicants and Other Substances	Select and produce/obtain cDNA and RNA probes for detecting changes in message RNA (mRNA) levels for the various neurogrowth/ neurotrophic (NTFs) which are likely to be involved in either secondary mechanisms of neurotoxicity or repair after neurotoxicant insult. Detect changes in NTF mRNAs after insult to neurotoxicants and other substances, and determine if these are the same for young and older animals.
E0692701 E0692711	**Dobrovolsky** Heflich	Active/ **Gen Tox/** METH	Development and Validation of Mouse Embryonic Stem Cell Cultures for use in Generating Animal Models with Targeted Transgenes (CDER) (CBER)	Mouse ES (Embryonic Stem) cell lines will be established; The ability of ES cell lines to contribute to the germ line of mice will be determined.
E0692801	**Evans** Komoroski Mrak	In Review/ **Bio Tox/** CNPT	Metabolite Changes in Alzheimer Disease (AD) by ^{1}H NMR	Using high resolution of ^{1}H NMR of methanol/water extractors, we will compare the metabolite profiles in four key regions of the brain for 36 patients, definitively diagnosed with AD by postmortem neuropathologic assessment, with those of 24 controls, matched for age and postmortem interval. Within the AD group, we will correlate the concentrations of the various metabolites with the severity of the disease.

Project Number	Principal/ Co-Principal Investigator(s)	Status/ Res. Area/ GOAL	Title	Objective
E0692901	**Terry** Hansen Streck	Active/ **R&D Tox/** PRED	Toxicant Effects on Neural Cell Adhesion Molecule and N-Cadherin during Mouse Neural Tube Closure (CFSAN)	To determine the optimum time of neural cell adhesion molecule (NCAM) and N-Cadherin expression in the closing CD-1 mouse neural tube; to quantitate changes in neural fold NCAM and N-cadherin levels following embryonic exposure to valproic acid, lithium or heat *in vivo*.
E0693001	**Scallet** Ali Hall Johannessen Paule Rountree Sandberg Schmued Slikker Sobotka	Active/ **Neuro Tox/** AGNT	Estimating Quantitative Neurotoxicity Risk from Domoic Acid Exposure (CFSAN)	To correlate pharmacokinetic profiles of single and multiple doses of domoic acid with associated neurohistological and behavioral effects in non-human primates; To identify genetic factors modulating domoic acid sensitivity in Wistar rats; To identify neurochemical biomarkers of domoic acid exposure and damage.
E0693101	**Wilkes** Cairns Chen Fry Heinze Kaysner Lay Miller Rafii Sutherland Turturro Voorhees	Active/ **Chemistry/** KNLG	First Phase Development of a Rapid Screening Method for Identification of Complex Mixtures by Pyrolysis-Mass Spectrometry with Computerized Pattern Recognition (CFSAN) (ORA)	Evaluate feasibility of the application of pyrolysis mass spectrometry (PyMS) with computerized pattern recognition (PattRec) for the rapid identification of a sample (a) which is a complex chemical mixture, (b) which is a member of a set of such mixtures, and (c) for which there is a regulatory need to distinguish the individual members of the set. Typical examples of applications: (a) the rapid identification of culturable pathogenic and non-pathogenic bacteria in food, (b) the distinction of adulterated from pure foods or cosmetics, or of generic from brand name pharmaceutical products, or (c) demonstrating the virginity of plastic materials used in food containers.
E0693201	**Rafii** Cerniglia	Active/ **Micro/** METH	Polymerase Chain Reaction Fragment Length Polymorphism (PCR-RFLP) for Analysis of the Azoreductase Gene of Anaerobic Bacteria Isolated from the Human Intestinal Tract	To study the effects of genetic variations on the metabolic activities of azoreductases from bacteria isolated from human intestinal tract.

Project Number	Principal/ Co-Principal Investigator(s)	Status/ Res. Area/ GOAL	Title	Objective
E0693301 E0693311 E0693321 E0693331	**Dass** Casciano Harris Heflich Manjanatha	Active/ **Gen Tox/** PRED	Tumor Prone P53-Deficient Transgenic Mice (TSG-p53TM): A Potential System to Augment the Sensitivity of Carcinogenicity Testing and for Studying the Mutational Basis of Tumors (CDER) (CBER)	The genome instability of p53-deficient mice will be determined by monitoring the frequency of spontaneous mutations in the hprt biomarker gene of T-lymphocytes from the spleen. The time for appearance of tumors in the p53 heterozygotes will be compared with that for the wild type mice; ras and p53 mutations will be examined in such tumors. The frequency of mutations that arise on exposure of these animals to the carcinogens benzo[a]pyrene and dimethylnitrosamine in a neonatal carcinogenicity protocol will be monitored at the hprt locus in T-lymphocytes. The spectrum of carcinogen-induced mutations in the hprt locus will be determined by PCR and DNA sequencing; this information may indicate mutational mechanisms, serve as a fingerprint of environmental exposure, and permit risk assessment.
E0693501	**Parsons** Heflich	Active/ **Gen Tox/** METH	Development of Methods for the Biochemical Selection of Mutations	Establish biochemical selection methods to detect and quantify rare mutations in the DNA or mutagen-treated animals. The value of the E. coli mismatch binding protein (Muts), used with the polymerase chain reaction (PCR), as a biochemical selection for mutations in the ras oncogene will be evaluated.

Project Number	Principal/ Co-Principal Investigator(s)	Status/ Res. Area/ GOAL	Title	Objective
E0693601 E0693611	**Ang** Churchwell Doerge Freeman Hansen Luo Thompson	Active/ **Chemistry/** METH	Development of Analytical Methods for Determination of Amoxicillin and Lincomycin in Fish Tissues (CVM)	Develop highly sensitive analytical methods utilizing reversed-phase HPLC or GC for determining trace levels of amoxicillin and lincomycin residues in fish tissues. Specifically, the goal is to develop analytical methods which can be applied to determine amoxicillin in catfish muscle tissue and salmon muscle and skin tissues at 10 ppb and to determine lincomycin in salmon muscle and skin tissues at 100 ppb as suggested by the FDA Center of Veterinary Medicine (CVM). Analytical residues in both the catfish and salmon tissue substrates will be developed.
E0693801	**Evans** Hanna	Active/ **BioTox/** METH	Quantitative Determination of Enantiomers Composition and Purity of Drugs by NMR Spectroscopy (ORA)	1) To develop NMR methods to monitor enantiomeric purity of a group of B-adrenergic antagonists (i.e., propranolol, sotalol, pindolol, and timolol.) The hypothesis is that effective NMR methods can be developed to monitor the enantiomeric purity of these drugs; 2) To develop NMR methods to monitor degradation products of a coronary vasodilator (nifedipine). The hypothesis is that effective NMR methods can be developed to monitor the degradation products of this drug.
E0694201	**Zhang** Ali Cerniglia Evans Freeman	Active/ **Micro/** PRED	Microbial Transformations of Antidepressants	To establish a microbial system with a broad range of biotransformations as a model for mammalian drug metabolism of psychoactive compounds.

Project Number	Principal/ Co-Principal Investigator(s)	Status/ Res. Area/ GOAL	Title	Objective
E0694301 E0694311 E0694321	**Frederick** Ali Binienda Clausing Gillam Paule Slikker	Active/ **Neuro Tox/** AGNT	Behavioral and Neurochemical Effects of Short Course, High Dose Exposure to Methylenedioxymethamphetamine (MDMA) or dexfenfluramine (FEN) in Rhesus Monkeys	1) To establish acute dose-response curves for MDMA and d-FEN using performance of two groups of rhesus monkeys in the NCTR primate operant test battery (OTB). 2) To produce long-term damage to the serotonin (5-HT) system of the forementioned monkeys via short course, high dose administration of MDMA or d-FEN. 3) To determine whether rhesus monkeys exposed to short course, high dose MDMA or d-FEN exhibit persistent changes in CNS functioning, as quantified by changes in OTB performance. 4) To determine if short course, high dose exposure to MDMA or d-FEN produces long-lasting changes in CNS function by establishing a second acute dose-response curve for each drug after each exposure. 5) To demonstrate possible long-term changes in both neurochemical and behavioral endpoints resulting from MDMA and d-FEN exposure in rhesus monkeys that may assist in the determination of the status of these drugs as therapeutic agents.
E0694501	**Doerge** Holder	Active/ **Chemistry/** METH	Development of Methods for Analysis and Confirmation of B-Agonists (CVM)	1) Develop determinative and confirmatory procedures using LC-APCI/MS for multiresidue screening B-agonists in livestock tissues. 2) Develop synthetic procedures to produce authentic B-agonist standards for use in regulatory screening. 3) Explore the use of packed column supercritical fluid chromatography (SFC) coupled to APCI/MS as a more efficient technique for chromatographic separation in the screening of large numbers of B-agonists in livestock issues.

Project Number	Principal/ Co-Principal Investigator(s)	Status/ Res. Area/ GOAL	Title	Objective
E0694601	**Kadlubar** Anderson Potter	Active/ **Mol Epi/** PRED	A Case-Control Study of Pancreatic Cancer and Aromatic Amines	To measure the associations of aromatic amine exposure and metabolism with the risk of pancreatic cancer. The sources of aromatic and heterocyclic amines to be studied are cigarette smoking and diet; the metabolic capabilities to be studied are acetylator status and N-oxidation status.
E0694701	**Kadlubar** Lang	Active/ **Mol Epi/** PRED	Role of Acetylation and N-Oxidation in Colorectal Cancer	To confirm the initial findings of our pilot study regarding the roles of heterocyclic amine metabolism and exposure as putative risk factors from the diet or the environment. The sources of heterocyclic amines to be studied are cigarette smoking, diet and cooking methods; the metabolic pathways to be studied include heterocyclic amine N-oxidation status and O-acetylation status.
E0694801	**Dass** Casciano Heflich	Active/ **Gen Tox/** PRED	*In Vitro* Induction of Mutation by Carcinogens in the HPRT Gene in Mouse T-Lymphocytes (CDER) (CBER)	1) T-lymphocytes will be isolated from the spleen of (unexposed) mice following published procedures. 2) Mutants defective in the hprt gene (thioguanine-resistant or TG) will be isolated by a limiting dilution technique. 3) Mutations in the hprt gene will be sequenced.

Project Number	Principal/ Co-Principal Investigator(s)	Status/ Res. Area/ GOAL	Title	Objective
E0694901 E0694911	**Pipkin** Hinson Lyn-Cook Manjanatha Shaddock	Active/ **Gen Tox/** PRED	The Effect of P53 Null Phenotype on Bleomycin-induced Stress Protein Elicitation *In Vivo* in Transgenic Mice (CDER) (CBER)	1) Investigate the structure of the sp 70 and 90 genes by Southern blot in the 8-10 week old p53 null mouse in comparison with C57BL/6 control mouse; 2) Investigate the stress protein (SP) metabolic turnover (synthesis 35S-labeling) as a reflection of gene expression in the control homozygous C57BL/6 (+/+) and the null p53 homozygous TSG (-/-) mice as elicited by bleomycin (BL) at 1,2,3,4,and 5 months of age (during the G1-phase of the cell cycle) by polyacrylamide gell electrophoresis (PAGE), and their levels of radio-labeling calculated by computerized electronic area measurements. If stress proteins (sps) are absent in bone marrow nuclei of 1 month old p53 null mice (sp synthesis is dependent on the presence of the p53 gene) or if their expression is below the level of measurement then the protocol will be discontinued at test group 1. 3) Investigate the phosphorylation patterns of sps as a reflection of gene expression as elicited by BL as in objective 1). 4) To identify and examine nuclear polypetides other than sps for synthesis and phosphorylation levels as possible biomarkers of metabolic alterations and gene expression during phases of the celll cycle in control and homozygous p53 null mice following administration of BL.

Project Number	Principal/ Co-Principal Investigator(s)	Status/ Res. Area/ GOAL	Title	Objective
E0695001	**Manjanatha** Casciano Harris Shaddock	Active/ **Gen Tox/** PRED	Molecular Analysis of *In Vitro* Mutations in the Transgenic Rat2 Cells Exposed to DMBA and Tamoxifen: Comparison of Mutagenesis in the Transgene lacI with the Endogenous Gene hprt (CDER) (CBER)	1) To determine the mutant frequency and mutation spectrum of the lacI transgene in Rat2 cells following exposure to DMBA and tamoxifen prior to evaluation in Blue Rat. 2) To determine the mutant frequency and mutations spectrum of the endogenous hprt reporter gene in Rat2 cells following exposure to DMBA and tamoxifen. 3) Compare *in vitro* mutant frequencies and mutational spectra with those determined in the Big Blue rats *in vivo* from Experiment 6906.
E0695201	**Chou** Jackson James-Gaylor Poirier	In Review/ **Bio Tox/** CNPT	Effects of Dietary Restriction on the Post-Initiation Stages in Aflatoxin B1 Induced Carcinogenesis on Male F-344 Rats fed Methyl Deficient Diets (CFSAN)	To study the interactions of dietary restriction (DR) and methyl deficiency (MD) on the alterations of hepatic oxidative DNA damages, DNA methylation, cell proliferation, oncogene and tumor suppressor gene mutation, preneoplastic foci formation and tumor incidence during the post-initiation stages of AFB1-induced carcinogenesis in male F344 rats. The results of these studies will : 1) test the hypothesis that DR may be an antagonist to the promotional effect of MD in the AFB1-induced carcinogenesis; and 2) evaluate the correlations between the effects of DR and MD on the chemically induced tumor incidence and various biomarkers during the post-initiation stages of carcinogenesis.
E0695301	**Young** Bolon Branham Haas Meehan Sheehan Warbritton	Active/ **Biometry/** PRED	Rodent Embryo and Fetal Sectioning for Three Dimensional Image Reconstruction and Animation	To develop the staining and sectioning techniques for conventional and laser scanning confocal microscopy to produce electronic images of rodent embryos and fetuses that can be used for computerized image morphing, 3D reconstruction, and animation.

Project Number	Principal/ Co-Principal Investigator(s)	Status/ Res. Area/ GOAL	Title	Objective
E0695401	**Freni**	Active/ **Biometry/** CNPT	Resting Metabolic Rate, Body Composition, and Dietary Assessment	1) Develop a validated prediction model for the resting metabolic rate; 2) Collect dietary intake data while maximizing their accuracy; 3) Identify under- or over- reporting of dietary intake data, with emphasis on potential relations between reporting bias and anthropometric data.
E0695501	**Griffin** Gollon Hobbs Kadlubar	Active/ **Tech Adv*****/** AGNT	Accumulation of Manganese in Edible Muscle of Channel Catfish (Ictalurus punctatus) Following Exposure to Water (CVM)	To determine the concentration and retention time of residual manganese in edible muscle of channel catfish after exposure to water borne potassium permanganate.
E0695601	**Jackson** Weis	Active/ **R&D Tox/** CNPT	An Evaluation of Dietary Fibers for the Prevention of Mammary Cancer in Female Rats	1) To develop an assay using 14C-estradiol to determine the amount of estrogen excreted via the feces by animals maintained on diets containing different types and levels of dietary fibers 2) Using this assay, to evaluate several dietary fibers for their ability to increase estrogen excretion and to lower estrogen levels; 3) To test the most effective fibers in the DMBA-mammary tumor model for their ability to inhibit tumor development at dietary levels shown to lower estrogen levels; 4) To establish if the inhibitory effect of dietary fiber on mammary tumor inhibition is dependent on the level of dietary fat.
E0695701 E0695711	**Young** Fleisher Laborde Young	Active/ **Biometry/** AGNT	Changes in the Disposition of Methadone in Pregnant Rats and their Fetuses	To conduct pharmacokinetic experiments in the non-pregnant, pregnant, and post-partum rat in order to quantify the differences in disposition of methadone.

*****Technology Advancement

Project Number	Principal/ Co-Principal Investigator(s)	Status/ Res. Area/ GOAL	Title	Objective
E0695801 E0695811	**Chen** Aidoo Casciano Heflich Manjanatha Mittelstaedt	Active/ **Gen Tox/** PRED	Mutant Frequencies and Types of Mutations Induced by Rat Carcinogens in the hprt and lacI genes of Big Blue Fischer 344 Rats (CDER) (CBER)	1) To determine the mutant frequencies at the endogenous reporter gene hprt in T-lymphocytes from the spleens of Fischer 344 rats following exposure to five mutagens: Aflatoxin B1, N-hydroxy-2-acetylaminofluorene, benzo(a)pyrene, 2-amino-3-dimethylimidazoquinoline, and tris(1-aziridinyl)phosphine sulfide; 2) Determine the mutant frequencies at the endogenous gene hprt and exogenous gene lacI from transgenic rats exposed to a mutagen selected from the five compounds examined in Objective 1; and 3) Determine the types of mutations produced in the hprt and lacI genes in the mutants induced in Objective 2.
E0695901	**Rafii** Cerniglia Hehman	In Review/ **Micro/** METH	Cloning and Characterization of the Genes Involved in the Metabolism of Nitro Compounds by Mycobacterium sp. Pyr-1	To understand the substrate specificity, cofactor requirement, and molecular characteristics of Mycobacterium sp. Pyr-1 nitroreductase and to determine the relationship of this enzyme to other microbial and mammalian nitroreductases involved in reduction of therapeutic nitro compounds.
E0696001	**Hansen** Dial Grafton Terry	Active/ **R&D Tox/** PRED	Further Studies on the Mechanism of Valproic Acid Acid-Induced Embryotoxicity (CFSAN)	1) Determine a sensitiive period for VPA-induced neural tube defects (NTDs) in rat embryos treated *in vitro*; 2) Determine if VPA produces hypomethylation of DNA in treated rat embryos *in vitro*; 3) Determine S-adenosylmethionine/S-adenosylhomocysteine (SAM/SAHC) ratios in control and VPA-treated embryos during the sensitive period; 4) Determine if VPA produces hypomethylation of DNA in embryos treated with the drug *in vivo*; 5) Determine if inactivation of methionine synthase increases the embryotoxicity of VPA.

Project Number	Principal/ Co-Principal Investigator(s)	Status/ Res. Area/ GOAL	Title	Objective
E0696101 E0696111	**James-Gaylor** Miller Pogribny	Active/ **Bio Tox/** AGNT	Mechanisms of Immunotoxicity and Carcinogenicity Associated with Silicone Breast Implants (CDRH) (OWO)	Examine the acute and chronic cellular and subcellular responses to subcutaneous silicone implants utilizing state-of-the-art immunohistochemistry and molecular biology technologies.
E0696201	**Hammons** Blann Kadlubar Lyn-Cook	Active/ **Mol Epi/** PRED	Methylation Profile, Gene Expression, and Enzyme Activity of CYP1A2 in Human Livers	To determine the possible involvement of epigenetic mechanisms in the regulation of the CYP1A2 gene. DNA methylation profiles of this gene will be examined and compared with gene expression and enzyme activity in human livers that have been classified by smoking status, gender, and age.
E0696301	**Delclos** Chen Colvert Eaton Klimberg	Active/ **Bio Tox/** AGNT	Sexual Dimorphism in the Inflammatory Response to Biomaterials	Determine if a sex difference in the *in vitro* response of human monocytes and mouse peritoneal macrophages to various biomaterials can be demonstrated. Based on existing literature, we hypothesize that there will be a significant sex difference in the synthesis and release of inflammatory mediators that could influence the biocompatibility of the material.
E0696401 E0696411 E0696431	**Wolff** Cooney James-Gaylor Pogribny	Active/ **Bio Tox/** CNPT	Prevention of Ubiquitous Synthesis of the Agouti Protein by Methyl Supplemented Diet	To test the hypotheses that dietary methyl supplements fed to pregnant mice: 1) Affect selected aspects of DNA methylation and phenotypic characteristics dependent on DNA methylation patterns in the offspring; 2) increase the proportion of methylated cytosines in IAP promoter sequences in Avy/a offspring; and 3) increase expression of the agouti phenotype and have no gross morphological effects on the offspring.

Project Number	Principal/ Co-Principal Investigator(s)	Status/ Res. Area/ GOAL	Title	Objective
E0696501	**Erickson** Campbell Holland	Active/ **Micro/** METH	Development of an Improved Method for Determining the Tuberculocidal Activity of Chemical Disinfectants for Medical Devices	To develop an improved method for the rapid and accurate evaluation of the tuberculocidal activity of chemical disinfectants and sterilants. The hypothesis is that molecular methods can be used to (a) improve quantitation of the disinfectant activity, (b) improve the reliability of the assay, and (c) shorten the time required for testing in comparison with the standard culture techniques. This protocol addresses the NCTR strategic research goal of conduct method-, agent- , or concept-driven research, through satisfying the need for an analytical method to accurately evaluate these products.
E0696701	**Littlefield** Chou Hass Mikhailova	Active/ **Bio Tox/** AGNT	Investigations into the Interactive Oxidative Effects of Magnesium and Calcium with Selected Heavy Metals	To evaluate the influence of magnesium and calcium, both alone and in combination, on the toxicity from selected heavy metals in respect to the induction of oxidative DNA damage; To investigate the occurrence of adaptive responses in respect to the occurrence of oxidant stress from heavy metal toxicity; To evaluate interactions of the anti-oxidant ascorbate in respect to oxidative damage from selected heavy metals; To gain insight into mechanisms of action in regard to the toxicity and tumorigenic process instigated by heavy metals.
E0696901	**Baker** Medlock Sheehan	Active/ **R&D Tox/** KNLG	Enxymatic Oxidation of 17β-Estradiol: Role of the Products in Hormone Action (CFSAN)	To determine how estradiol metabolites formed by peroxidase or tyrosinase interact with the estrogen receptor.
E0697001 E0697011	**Coles** Beland Fullerton Heflich	Active/ **Bio Tox/** CNPT	Sequence Specificity of DNA Adduct Formation and Removal Following Chronic Carcinogen Administration	To determine whether or not certain nucleotide sequences bearing carcinogen adducts are more resistant than others to DNA adduct formation and repair, and to identify these sequences.

Project Number	Principal/ Co-Principal Investigator(s)	Status/ Res. Area/ GOAL	Title	Objective
E0697101	**Ahn** Chen	Active/ **Biometry/** CNPT	Tree-Structured Over-Dispersed Binomial and Over-Dispersed Poisson Regression Models	1. To develop a tree-structured regression model to analyze over dispersed binomial and over dispersed Poisson data; 2. To develop an algorithm that extends tree-structured regression to the generalized linear regression model; 3. To identify the local effect of the covariates by stratified analysis of the data using tree-structured models; 4. To apply this method to developmental toxicity studies.
E0697201	**Wilkes** Abramson Billedeau Freeman Heinze Pothuluri	Active/ **Chemistry/** METH	Universal Interface Development and Applications (ORA)	The ultimate objective of this work is to develop a variety of new technologies for improving high performance liquid chromatography (HPLC) detection. By eliminating hazards associated with radioactivity, it can make possible metabolic drug studies involving human subjects. Several CRADAs will be negotiated during the work to facilitate development of commercial versions of the devices which show the most promise.
E0697301	**Streck** Branham Sheehan	Active/ **R&D Tox/** KNLG	Mechanism of Tamoxifen Developmental Toxicity and Neoplasia: Tamoxifen Effects on the Rat Uterine Insulin Like Growth Factor System (CDER) (OWO)	1) To define the ontogeny of insulin-like growth factor (IGF) system mRNA expression in the developing rat uterus; 2) To determine the uterine cell types in which IGF system mRNAs are expressed; 3) To determine the effects of diethylstilbestrol (DES), tamoxifen (TAM), and ICI 182,780 on IGF system mRNA expression at selected developmental stages.
E0697401	**Arani** Chen Freni	Active/ **Biometry/** KNLG	Collinearity Under Proportional Hazards Model (CDER)	1) To provide diagnostic tools to detect the presence of collinearity under proportional hazards and its quantitative effect on the results; 2) To provide algorithms to combat the harmful influence of collinearity, i.e., stabilize the parameter estimates and their variance. 3) To conduct a simulation study to examine the effectiveness of the algorithms.

Project Number	Principal/ Co-Principal Investigator(s)	Status/ Res. Area/ GOAL	Title	Objective
E0697501 E0697511	**Aidoo** Bishop Heflich Lyn-Cook Mittelstaedt	Active/ **Gen Tox/** PRED	The Frequency and Types of Spontaneous Mutations Found in the hprt and lacI Genes of Lymphocytes from Transgenic Big Blue Rats (CDER) (CBER)	1) To determine the frequency of spontaneous mutation at the hprt and lacI loci in pre-weanling, young (four-month-old) and old (18-month-old) Big Blue rats; 2) To determine the types of mutations present in the mutants from Objective 1; 3) To compare the results of the analysis conducted from Objective 1 and 2 to determine how well mutational results from the transgenic lacI locus predict mutations at the endogenous locus; 4) To compare the results of the hprt analysis conducted for Objective 1 and 2 with the results of similar analyses of spontaneous hprt lymphocyte mutations conducted in humans to determine how well mutational analysis in the model rat assay predicts mutagenesis in humans.
E0697601	**LaBorde** Hansen Hinson Lyn-Cook Pipkin Shaddock	Active/ **R&D Tox/** CNPT	Dose-Response of Retinoic Acid-Induced Stress Protein Synthesis and its Correlation with Developmental Toxicity in CD-1 Mice (CDRH)	Determine the incidence of limb malformations on gestation day 17 (GD 17) and the extent of synthesis of Sps in limb bud tissue determined 2.5 hr after RA treatment following various doses of RA administered on GD11. Determine the incidence of cleft palate on gestation day 17 (GD 17) and the extent of synthesis of Sps in craniofacial tissue determined 2.5 hr after RA treatment following various doses of RA administered on GD 13.

Project Number	Principal/ Co-Principal Investigator(s)	Status/ Res. Area/ GOAL	Title	Objective
E0697701	**Chen** Burkhart Casciano Heflich Malling	Active/ **Gen Tox/** PRED	Evaluation of Chemical-Induced Mutagenesis in Transgenic Mice Containing the ΦX174 am3 (CDER) (CBER)	Establish the experimental parameters necessary to demonstrate a mutant frequency of 1.5 to 2 fold above background; Establish the sensitivity of the am3 mouse model to carcinogens and germ-cell mutagens expected to produce DNA damage at A:T base pairs. Where possible, compare the sensitivity of the ΦX174 system with that of other *in vivo* mutational systems; Establish several basic properties of the ΦX174 am3 assay by determining the tissue or organ specificity of responses to certain carcinogens and by determining the patterns of mutations detected by the assay.
E0697801	**Ambrosone** Kadlubar Tang Carino	Active/ **Mol Epi/** PRED	Chemical Carcinogenesis: Epithelial Cells in Breast Milk	1) To develop and refine a methodology for separation of luminal epithelial cells from human breast milk for DNA extraction; 2) To detect and quantify aromatic/hydrophobic-DNA adducts in luminal epithelial cells derived from human breast milk; 3) To detect genetic polymorphisms in carcinogen-metabolizing genes derived from DNA extracted from epithelial cells in human breast milk; 4) To evaluate the relationships between carcinogen-DNA adducts and smoking status, and adduct levels with polymorphisms in NAT1, NAT2, CYP1A1, and GSTM1.

Project Number	Principal/ Co-Principal Investigator(s)	Status/ Res. Area/ GOAL	Title	Objective
E0697901	**Frederick** Gillam Paule	Active/ **Neuro Tox/** PRED	Validation of the NCTR Nonhuman Primate OTB as a Predictor of Neurobehavioral Toxicity II. Pharmacological Manipulation at Specific Neurotransmitter Receptor Subtypes	1) To further explore the extent to which the use of operant behavioral techniques in nonhuman primates can serve to reliably model the effects of compounds selected to act on specific neurotransmitter systems; 2) To determine the acute dose-effect relationships of several drugs believed to act primarily at subtypes of specific neurotransmitter receptors using rhesus monkey OTB performance; 3) To characterize the relative sensitivities of the various behavioral endpoints in the NCTR OTB to pharmacological manipulation of specific neurotransmitter systems and to add new tasks to the NCTR OTB; 4) To more thorougly characterize the role of specific neurotransmitter systems in the expression of complex brain functions through the pharmacological manipulation of specific receptor subtypes of some of the known major neurotransmitter systems; 5) To determine if the acute behavioral effects of the exogenous compounds of interest differ with regard to gender in the rhesus monkey.

Project Number	Principal/ Co-Principal Investigator(s)	Status/ Res. Area/ GOAL	Title	Objective
E0698001	**Hansen** Ang Churchwell Doerge Luo Thompson Wilkes	Active/ **Chemistry/** METH	Development of Methods for Analysis and Confirmation of Erythromycin A Residues in Tissue Samples from Terrestrial and Aquatic Farmed Animals by Liquid Chromatography (CVM)	The principal objective of this project is to develop determinative and confirmatory analytical chemical procedures, using high performance liquid chromatography/ electrochemical detection, and high performance liquid chromatography/ atmospheric pressure chemical ionization mass spectrometric detection, for Erythromycin A in biological samples taken from agricultural animals. Specifically, the goal is to develop complete methods for the analysis of Erythromycin A in muscle and liver tissues from poultry, non-processed bovine milk, and muscle tissues from salmon, catfish, and shrimp. Sensitivity levels for these methods are expected to be at least 100 parts per billion for liver tissue and 50 parts per billion for muscle tissue and milk as requested by the Center of Veterinary Medicine.
E0698101	**Cooney** Poirier Wise	In Review/ **Mol Epi/** CNPT	Investigation of Short Term Dietary Methyl Supplementation in Manipulation of DNA Methylation and Methyl Metabolism in Mice	To determine whether short term dietary methyl supplementation in mice will effect qualitative or quantitative changes in levels of methyl metabolites, levels of DNA methylation or levels of cell proliferation or apoptosis. The effects will be determined at two time points and three levels of methyl supplementation. The studies proposed herein will provide data on some molecular and cellular events resulting from methyl supplemented diets. These studies will provide specific new data and use new test strategies that will help us better extrapolate between human and animal data.

Project Number	Principal/ Co-Principal Investigator(s)	Status/ Res. Area/ GOAL	Title	Objective
E0698201	**Allen** Siitonen Thompson	Active/ **Chemistry/** METH	Development of Multi-Residue Method(s) for the Determination of Lead and Other Metals in Sweeteners and Edible Oils by Plasma Atomic Emission Spectrometry (CFSAN) (ORA)	Develop method(s) on inductively coupled plasma atomic emission spectroscopy (ICP_AES) for the determination of lead in sweeteners and edible oils at proposed regulatory levels. Methods will be amenable to direct implementation in other laboratories and provide a reduction in time and cost as compared to existing methods.
E0698301	**Ali** Duhart Hussain Klein Lipe Mukherjee Newport Rountree Sandberg Scallet Schmued Slikker Ye	Active/ **Neuro Tox/** AGNT	Effects of Ibogaine on Neurotransmitter Systems, Generation of Free Radicals and Nitric Oxide Synthase Activity: Correlation with Neurohistological Evaluations in Mouse and Rat Brain (CDER)	1. To determine the effects of ibogaine on dopamine, serotonin and their metabolite concentrations in different regions of mouse and rat brain. 2. To determine the effects of ibogaine on reactive oxygen species (ROS) and lipid peroxidation in different regions of mouse and rat brain. 3. To determine the effects of ibogaine on the activities of several antioxidant enzymes superoxide dismutase, catalase,glutathione peroxidase and gluthathione levels in different regions of mouse and rat brain. 4. To evaluate the effects of ibogaine on the activity of nitric oxide synthase (NOS) in different regions of the mouse and rat brain. 5. To determine the levels of ibogaine, noribogaine and neurohormone, prolactin and corticosterone in plasma of mouse and rat. 6. To evaluate the neurohistorical effects of ibogaine in different brain regions in the mouse and rat and to correlate them with any neurochemical alterations.

Project Number	Principal/ Co-Principal Investigator(s)	Status/ Res. Area/ GOAL	Title	Objective
E0698401	**Chen** Kodell Zheng	Active/ **Biometry/** METH	Statistical Analysis and Characterization of the Joint Actions of Toxicants	To develop a procedure for analyzing the quantal response data from a mixture experiment at a fixed total concentration; to develop a procedure for analyzing the survival data from a mixture experiment at a fixed total concentration; To develop a mixture model including both proportions and total concentrations; to apply the proportion-concentration model to characterize the joint actions of toxicants.
E0698501	**Hansen** Dial Grafton	Active/ **R&D Tox/** PRED	The Role of Reactive Intermediates in Carbamazepine-Induced Embryotoxicity (CDER)	To determine if the anti-oxidant, glutathione (GSH), is able to decrease CBZ-induced embryotoxicity in mouse embryos; To determine if inhibition of GSH synthesis by L-buthionine-(S<R)-sulfoximine (BSO) increases the embryotoxicity of CBZ; to determine if the antioxidative enzyme, superoxide dismutase (SOD) decreases CBZ induced embryotoxicity; to determine if the prostaglandin H synthase inhibitor, aspirin, decreased CBZ induced embryotoxicity; to determine if treatment with 12-o-tetrade-canoylphorbol-13-acetate (TPA) which activates the release of arachidonic acid decreases CBZ induced embryotoxicity; to determine if treatment with eicosatetraynoic acid (ETYA), an inhibitor of both prostaglandin H synthase and lipoxygenase decreases CBZ induced embryotoxicity.

Project Number	Principal/ Co-Principal Investigator(s)	Status/ Res. Area/ GOAL	Title	Objective
E0698601	**Delclos** Blaydes Sepai	Active/ **Bio Tox/** CNPT	Serum Albumin adducts as markers of exposure to toluenediamines from implanted polyurethane foam	The goal of this experiment is to determine whether this protein-associated toluenediamine accurately reflects exposure to free toluenediamines released from the polyurethane by leaching or by breakdown of the polymer rather than exposure to toluenediamine containing oligomeric breakdown products. In addition to allowing an evaluation of this methodology for the purpose of monitoring exposure to polymer degradation products, these data will be used to help interpret an ongoing study of TDA-plasma protein adducts in German women with polyurethane covered breast implants.
E0698701	**Roberts** Benson Doerge Gehring Newkirk	Active/ **Bio Tox/** METH	Tandem Immunochemical - Analytical Methods (CVM) (ORA)	Develop combined immunochemical and analytical chemical techniques to clean up complex matrices containing analytes of regulatory interest and provide detection at low concentrations with selectivity capable of providing structural confirmation.
E0698801	**Wang** Cao Cerniglia	Active/ **Micro/** METH	Development of a Universal Protocol for Detection and Identification of 13 Species of Foodborne Pathogens in Foods by Polymerase Chain Reaction (PCR)	Develop PCR methods for detection and identification of 13 species of foodborne pathogens; modification of the PCR methods to use same conditions including use of the same PCR Cycler machine, same annealing temperature, and the same buffer system; detection of the pathogens in various food samples by PCR; development of a universal protocol for the PCR detection of the 13 species of food-borne pathogens in foods; and further improve the PCR specificity and sensitivity, and increase the species including other pathogenic E. coli and other non-anaerobic foodborne pathogens.

Project Number	Principal/ Co-Principal Investigator(s)	Status/ Res. Area/ GOAL	Title	Objective
E0698901	**Billedeau** Churchwell Cooper Doerge Wilkes	Active/ **Chemistry/** METH	Development of Methods for Analysis of Volatile and Nonvolatile N-Nitrosamines in Relevant Cosmetics and Nitrite Cured Meat Products (CFSAN)	Develop methods for extraction, cleanup, and anlaysis of non-volatile N-nitrosamines in cosmetics and meat products using combined LC detection methods with confirmation by compatible MS ionization methods; investigate the applicability of LC-ESI/MS and /or LC-APCI/MS as a multiresidue, trace level, quantitative technique for anlaysis of volatile, semi-volatile, and non-volatile N-nitrosamines in these consumer products.
E0699001	**Tang** Kadlubar	Active/ **Mol Epi/** PRED	The Role of Human Cytochrome CYP1B1 in Drug Metabolism and Carcinogenesis	To elucidate the role of human cytochrome P450 1B1(CYP1B1) in drug metabolism and carcinogenesis. Specific aims are: to design and develop peptide-specific antibodies against human CYP1B1; determine the levels of CYP1B1 protein in various human tissues; evaluate CYP1B1 expression as a biomarker for tumorigenesis; identify CYP1B1 inducers among the most common drugs and carcinogens; identify CYP1B1 substrates, including the endogenous steroid hormones, as well as drugs and carcinogens known to be metabolized by the closely related cytochromes P450 1A1 and 1A2; find specific enzyme inhibitors for CYP1B1; develop a sensitive, convenient, and speicific assay method for CYP1B1 enzyme activity *in vitro*; and evaluate genetic polymorphism(s) for CYP1B1 as an epidemiological marker for cancer risk.

Project Number	Principal/ Co-Principal Investigator(s)	Status/ Res. Area/ GOAL	Title	Objective
E0699101	**Feuers** Aidoo Desai	Active/ **Gen Tox/** PRED	Influence of Dietary Restriction on Somatic Mutation and Antioxidant Enzymes Induced by Exposure of Female and Male Fischer 344 Rats to Bleomycin (CDER)	To determine the frequency of occurrence of lymphocytes bearing a mutant form of the hprt gene as an indicator of DNA damage in caloric restricted and in ad libitum rats following exposure to bleomycin; to determine how the activity of antioxidant enzymes such as catalase, glutathione peroxidase, and glutathione reductase relates to the mutant frequencies determined from the above objective; to determine the activity of the electron transport systems as an indicator of mitochondrial function during drug exposure; and to evaluate the integrity of mitochondrial DNA in Bleomycin treated rodents.
E0699201	**Patterson** Binienda Duhart Kim Lipe Slikker	In Review/ **Neuro Tox/** PRED	Validation Study of the Physiologically-Based Pharmaco-kinetic (PBPK) Model for Description of low-dose, long-term Exposure of 2,4-Dichlorophenoxyacetic Acid (2,4-D) Dosimetry in the Central Nervous System (CNS) (CFSAN)	To obtain CNS pharmacokinetic profiles of 2,4-D transport in the rat after low dose, chronic dosing (28 days). The data will be used to validate the previously developed PBPK model which simulates the uptake, distribution, and clearance of 2,4-D.
E0699301	**Delclos** Blaydes Chen Sams	Active/ **Bio Tox/** KNLG	Evaluation of Host Factors Contributing to Differences in the Response to Biomaterials (CDRH)	The goal of this protocol is to examine model systems that may be useful in the study of factors that regulate the extent of, and adverse effects arising from, the response to foreign bodies. As oxidative stress, including oxidative DNA damage, may play a major role in the foreign body reaction and in certain long-term adverse effects that may be associated with that reaction, we will also evaluate the utility of the air pouch model of inflammation to study species and strain differences in the development of and response to oxidative DNA damage.

Project Number	Principal/ Co-Principal Investigator(s)	Status/ Res. Area/ GOAL	Title	Objective
E0699401	**Gazzara** Dow-Edwards Gough Holson	In Review/ **R&D Tox/** CNPT	Efficacies and Relative Potencies of the Monoamine Uptake Inhibitors Cocain, Fluoxetine and GBR 12909 in Preweanling Male and Female Rats as Measured by Cerebral Microdialysis	To establish the efficacies of three different compounds as reptake inhibitors of extraneuronal dopamine and/or serotonin *in vivo* in the nucleus accumbens of preweanling male and female rats; to compare the relative potencies with which these compounds inhibit the reuptake of extraneuronal dopamine and/or serotonin *in vivo*; to determine whether or not age and/or sex are factors that alter the efficacies and relative potencies of these compounds as reuptake inhibitors of extraneuronal dopamine and/or serotonin *in vivo*.
E0699501	**Carino** Ambrosone Kadlubar McDaniel Tang	Active/ **Mol Epi/** PRED	Characterization of Ovarian-Specific Biotransformation of Estradiol: A Model for the Identification of Inter-individual Variability in Tissue Specific Steroid Metabolism	With the current widespread use of hormone based therapies and the increasing support for hormone based chemoprevention therapies for breast cancer, concern regarding the role of estrogens, anti-estrogens, and progesterones in the etiology of and/or progression towards cancers of hormonally-responsive tissues has continued to remain controversial in the cancer literature. Numerous studies, both epidemiological, as well as animal exposure studies, strongly suggest a role for estrogens in the carcinogenic cascade of several hormone responsive cancers. It is predicted that the identification of genetic variability in estrogen metabolism among individuals can be utilized as biomarkers ot assess cancer risk in large population based epidemiological studies, providing a toool to address more directly concerns regarding the association of estrogens, estrogen metabolites, hormonal based therapeutics, and carcinogenesis in the human population.

Project Number	Principal/ Co-Principal Investigator(s)	Status/ Res. Area/ GOAL	Title	Objective
E0699601	**Morris** Chen Domon McGarrity	Active/ **Gen Tox/** CNPT	Evaluation of the Genotoxic Potential of Genistein in Human Lymphoblastoid Cells	To confirm the potential mutagenicity of genistein utilizing the tk/hprt mutation assay; to determine if apoptosis can account for the toxicity of genistein; to characterize the effect of genistein exposure on the traverse of the cell-cycle; to evaluate the role of the p53 tumor suppressor gene in the response to genistein exposure by performing the experiments which address objectives 1,2,and 3 in both the AHH-1 tk(p53) and L3(tk;p53) human lymphoblastoid cell lines.
E0699701	**Miller** Freeman Heinze Holcomb Lansden Lay Thompson Wilkes	In Review/ **Chemistry/** METH	Innovative Methods for Determining Food Quality: Decomposition, Safety and/or Economic Fraud (ORA)	Examination of the TVB and PU, CD and HS methods for potential regulatory use (decomposition); develop novel rapid detection methods for the determination of analytes in seafood; extension of the microwave mediated distillation-solid-phase extraction (MD-SPE) technologies.
E0699801	**Hart** Aidoo Chou Duffy Feuers Fu Hass James-Gaylor Leakey Lu Lyn-Cook Pipkin Turturro	Active/ **Cal Res/** PRED	Memphis Study: Evaluation of Calorically Restricted Human Surgical Samples Received from Dept. Of Surgery, University of Tennessee, Memphis	Determine whether rodents and humans behave biologically in the same manner when calorically deprived but nutritionally supplemented.

Project Number	Principal/ Co-Principal Investigator(s)	Status/ Res. Area/ GOAL	Title	Objective
E0699901	**Kim** Cerniglia	In Review/ **Micro/** PRED	Biochemical and Molecular Analysis of Polycyclic Aromatic Hydrocarbon (PAH) Degradation by Bacteria	1. To characterize multiple genes for the initial aromatic dioxygenase from F. Yanoikuyae B1; 2. to determine putative common roles of ferrodoxin and reductase components of initial dioxygenase in mono- and polycyclic aromatic hydrocarbon degradation; 3. to determine roles of the NahD (2-hydroxychromene-2-carboxylate isomerase) and NahE (cis-o-hydroxybenzylidenepyruvate aldolase) in polycyclic aromatic hydrocarbon degradation by S. Yanoikuyae B1; 4. to determine molecular basis for polycyclic aromatic hydrocarbon degradation by Mycobacterium sp. PYR-1.
E0700001	**Weis** Jackson	In Review/ **Bio Tox/** PRED	Determination of Uracil Misincorporation and Abasic Sites (DNA Strand Breaks) in Rat Liver from Rats Fed Cereal Based, Purified, or Folate-Methyl Deficient Diets: Role of Dietary Nucleotides	1. to optimize an enzymatic assay for detecting the presence of misincorporated uracil and the increase of abasic sites in DNA using the excision-repair enzyme uracil DNA glycosylase; 2. to compare the level of DNA strand breaks arising from cereal-based diets with a semipurified diet, AIN-76A and with semipurified folate/methyl deficient diet; 3. to determine whether the dietary supplementation with preformed nucleotides will decrease the uracil misincorporation and abasic sites produced by these deficient diets.
E0700101	**Nawaz** Cerniglia Khan	In Review/ **Micro/** CNPT	Purification and Characterization of Antibacterial Protein from Oysters (CFSAN)	1. purification of the antibacterial protein from oyster homogenate; 2. physical, biochemical, immunological, and molecular characterization of the protein; 3. determination of the kinetics of the inhibitory reaction.

Project Number	Principal/ Co-Principal Investigator(s)	Status/ Res. Area/ GOAL	Title	Objective
E0700201	**Valentine** Burkhart Fane	In Review/ **Gen Tox/** PRED	The Development of Transgenic Mice Harboring Bacteriophage ΦX174 with Sites Specific for Detecting Mutations at Guanosine: Cytosine Nucleotides, Small Frameshifts, and Extended Deletions (CDER) (CBER)	To find specific mutations in bacteriophage ΦX174 that render the bacteriophage non-infectious and that will revert to plaque-forming ability only when mutation occurs by specific mechanisms: 1. base substitution at a G:C base pair or 2. frameshift caused by deletion of one or two nucleotides. An additional objective is to determine the feasibility of using ΦX174 to detect the deletion of an extended sequence. Phage harboring these mutations will be used to construct a transgenic mouse model for measuring mutations *in vivo*.
E0700301	**James-Gaylor** Hart Muskhelishvili Pogribny	In Review/ **Bio Tox/** CNPT	Nutritional Modulation of Apoptosis and Chemosensitivity: A Novel Anticancer Strategy	1. In NMU-initiated mammary epithelial cells, to determine whether nutritional manipulation of the cell cycle combined with low dose chemotherapy will permanently eliminate p53-independent and p53-dependent preneoplastic and neoplastic cells. 2. to determine the mechanisms of cell death induced by nutritional manipulation and low dose chemotherapy by examining molecular endpoints associated with p53-dependent and independent pathways of apoptosis.
E0960001	**Cooney** Poirier Wise	Active/ **Mol Epi/** AGNT	Preliminary Assessment of Methyl Supplements, Metabolism and DNA Methylation	Make a preliminary assessment of whether or not dietary methyl supplements in adult mice and rats will effect short term changes in levels of methylation metabolites or levels of DNA methylation. Dietary methyl supplements can signficantly improve methyl availability in humans even when the origin of reduced methyl availability is genetic. This project will also provide assessment of methodologies for collecting multiple animal organs for analysis of labile molecular components.

Project Number	Principal/ Co-Principal Investigator(s)	Status/ Res. Area/ GOAL	Title	Objective
P00268	**Flammang** Casciano Chen Freni Kodell	Active/ **Off Res******/** RSUP*******	NCTR/FDA Integrated Research (CFSAN) (CDER) (CDRH) (CVM) (CBER)	Interaction with and development of collaborative projects with other FDA Centers.
P00319	**Roberts** Partridge	Completed/ **Bio Tox/** METH	Develop Methodology to Test antibody Response in Modified Sheep RBCs	Receive treated (biotin(labeled)) sheep carcass, blood and tissue samples to develop methodology to test for antibody response to modified RBCs.
P00338	**Turturro**	Active/ **Cal Res/** METH	Training for Special Employment Program (Foreign National) in the PCR	To provide training for individuals in the Foreign National Training Program in the PCR.
P00339	**Ferguson**	Completed/ **R&D Tox/** PRED	Training in the Use of Rodents in Behavioral Toxicology and Teratology and Gross Dissection Techniques	This is one of the laboratory exercises planned for the 1994 AEGIS program which will introduce the students to the field of behavioral toxicology/teratology and the importance of behavior as an endpoint. The proposed exercise would require 17-20 rats (1/ student) in which each student would be responsible for 4 consecutive days of open field testing.
P00341	**Morris** Fogle Paule	Completed/ **Neuro Tox/** METH	Use of Rats to Test New Behavioral Equipment and Related Computer Software and to Train Personnel in Their Operation	1. To test the operation of new behavioral equipment and accompanying computer software designed to measure a variety of rat behaviors; 2. To train personnel in the operation of new behavioral equipment and accompanying computer software designed to measure a variety of rat behaviors.

******Office of Research

*******Research Support

Project Number	Principal/ Co-Principal Investigator(s)	Status/ Res. Area/ GOAL	Title	Objective
P00347	**Delclos** Sams	Completed/ **Bio Tox/** METH	The Rodent Air Pouch as a Model System for Evaluating Inflammation and DNA Damage Induced by Biomaterials	Evaluate a rodent air pouch model for assessment of the potential of biomaterials (materials used in medical devices) to induce inflammation and DNA damage. We plan to utilize this model in the future to study strain and species differences in the early response to biomaterials and the effect of surface modification on this response.
P00348	**Chen** Delclos	Completed/ **Bio Tox/** AGNT	*In Vitro* Granuloma Formation in Response to Biomaterials	Establish *in vitro* systems that will be utilized in future experiments to examine, at the cellular and molecular level, sex, strain, and species differences in the fibrotic response to various biomaterials.
P00351	**Ekuban**	Completed/ **Gen Tox/** METH	Training in Lymphocyte Mutagenesis and DNA Repair Assays for ORISE Visiting Scientist	Animals requested for use in training ORISE visiting scientist in the lymphocyte mutagenesis and DNA repair assays.
P00352	**Young**	Completed/ **R&D Tox/** CNPT	Pharmacokinetics/Pharmacodynamics in the Developing System and Impact on Risk Assessment	Investigator is part of the organizing committee for this symposium which will be held in Little Rock on April 21-26, 1996.
P00353	**Heflich**	Completed/ **Gen Tox/** METH	Rats for Training in Spontaneous Lymphocyte Mutation Frequencies	Requesting F344 rats to be used for training and practice in anticipation of beginning a planned protocol on spontaneous lymphocyte mutation frequencies in very young, young adult, and old rats.
P00354	**Shuttleworth**	Completed/ **Micro/** AGNT	Microbial Degradation of Dimethylformamide	To determine the metabolic pathway of dimethylformamide degradation in bacteria.
P00355	**Duffy** Zheng	Completed/ **Cal Res/** CNPT	Effect of Age and Dietary Restriction in the Peromyscus Leucopus Mouse	1) To place the long lived Peromyscus leucopus (PL) mouse on acute dietary restriction(DR); 2) To provide animals for training purposes in support of our foreign postdoctoral program.

Project Number	Principal/ Co-Principal Investigator(s)	Status/ Res. Area/ GOAL	Title	Objective
P00356	**Hass** Littlefield	Completed/ **Bio Tox/** METH	Determination of Viable, Necrotic, and Apoptotic Cells in Tumor Tissue	While volume can be used as a measure of tumor viability, it can also be misleading unless an estimate of necrosis is also considered. A better parameter of tumor size is viable cell number. The object of this experiment is to become familiar with flow cytometry methods that will measure viable, necrotic, and apoptotic cells in tumors from E0686501.
P00357	**Littlefield** Hass	Completed/ **Bio Tox/** AGNT	Do Heavy Metal Ions Induce Apoptosis?	Bivalent method ions are classified as essential or nonessential while some are both, depending upon the concentration. The above metals will be tested in the upper toxicity ranges to determine if the metals induce apoptotic responses.
P00358	**Sheehan** Branham Burroughs Medlock	Active/ **R&D Tox/** KNLG	Training in the Estrogen Developmental Toxicity Bioasssay (OWH)	This training protocol is the first phase of a project funded by the Office of Women's Health, FDA. In order to carry out the study, Pathology Associates, Inc. (PAI) need to be trained in animal sacrifice, tissue removal and processing, instrumentation, morphometric techniques, aspects of project management and procedures of data collection, recording, retrieval, reduction, and summarization.
P00363	**Prathibha** Bucci Lyn-Cook Mehendale	Completed/ **Mol Epi/** CNPT	Impact of Nutritional Modulation on Cell Proliferation of Human Hepatoma Cell Line (HuH 7)	Determine if glucose inhibits cell division by inhibition of DNA synthesis via alterations in protooncogene expression and growth factors modulation. Specific aims are to 1) develop an *in vitro* model using human hepatoma cell line to study hepatoxicity and 2) to investigate the molecular mechanisms of glucose-induced inhibition of proliferation of human hepatoma cells.
P00364	**Beland** Marques	Active/ **Bio Tox/** AGNT	DNA Adducts of Tamoxifen (OWH)	Preparation of a-hydroxytamoxifen and 4-hydroxytamoxifen synthetically.

Project Number	Principal/ Co-Principal Investigator(s)	Status/ Res. Area/ GOAL	Title	Objective
P00365	**Sams** Delclos	Active/ **Bio Tox/** METH	Oxidative Stress-Related DNA Damage and hprt Mutations in the Rodent Air Pouch	1) Developing a means of ensuring uniform exposure of the pouch lining to materials introduced directly into the pouch; 2) determining the time of maximal DNA damage; and 3) establishing conditions, based on the literature, for the culture of fibroblasts from the air pouch lining.
P00368	**Poirier**	Complete/ **Mol Epi/** PRED	A Literature Review of Secondary/Indirect Mechanisms of Tumor Development	Perform a thorough review of the available literature on agents that produce or promote cancer by indirect or secondary mechanisms (in contrast to direct alkylating agents) and to compile the information into a comprehensive review and evaluation of the available knowledge. The review will include both the identification of carcinogenic agents thought to act via indirect mechanisms and to identify and evaluate molecular and/or cellular mechanisms known or hypothesized to be responsible for the carcinogenic activity of these agents.
P00369	**Sheehan** Crews	Active/ **R&D Tox/** KNLG	A Biologically Based dose Response Model for Estradiol Effects on Sex Determination in Turtles	To experimentally test the hypothesis that if an endogenous hormone is causing an effect in a population, then treament with any dose of the same chemical cannot show a theshold because the threshold dose is already exceeded in the untreated animals.
P00370	**Norris** Baker Gaylor Harrison Lay Perkins Sheehan Shvets Strelitz Ulmer	Active/ **Dep Dir/** KNLG	Development of an Estrogen Knowledge Base for Research and Regulation	The purpose of this effort is to identify active elements in estrogen and estrogenic compounds, using the data in the NCTR estrogen database and commercial analysis and modeling tools. The application of traditional and advanced QSAR techniques to this ideal data set should either confirm the existence of active moieties or identify confounding factors that point the way towards further research. The result of this effort will be an estrogen database with predictive capability.

Project Number	Principal/ Co-Principal Investigator(s)	Status/ Res. Area/ GOAL	Title	Objective
P00371	**Beland**	Active/ **Bio Tox/** AGNT	*In Vivo* DNA Adduct Standards	A major breakthrough in the detection of DNA adducts was made in the early 1980's with the development of the 32P-postlabeling technique. The NCTR in collaboration with the EPA and IARC organized two international trials to analyze DNA samples using this technique. In this, the third trial, the synthetic standards will be used to quantify "unknown" *in vivo* samples. These "unknown" samples will be prepared in this project.
P00376	**Hansen** Streck	In Review/ **R&D Tox/** PRED	Development of Limb Bud Culture System	To develop a limb bud organ culture system for future molecular biology experiments - this project is a prelude to proposed project X70003.
P00377	**Aidoo** Montgomery	Active/ **Gen Tox/** AGNT	Training for Mutagenesis Studies for ORISE Internship Program Participant	Training program to enable ORISE program participant to conduct mutagenesis studies with four food derived mutagens.
S00006 E0002200 E0010900 E0011000 E0011100 E0011200 E0011300 E0014500 E0023500	**Cerniglia**	Active/ **Micro/** RSUP	General Microbiological Support - Bacteriology, Parasitology, Mycology, and Virology	To provide general microbiological support services.
S00007	**Doerge** Churchwell Heinze Lay Miller	Active **Chemistry/** METH	General Chemistry Support	To provide general analytical support such as feed analysis , stability and purity, etc.
S00009	**Gaylor** Chen Kodell	Active/ **Biometry/** CNPT	General Biometry Support	To assist NCTR scientists on the statisitcal design, analysis, and interpretation of toxicological experiments.
S00031	**Warbritton**	Active/ **Res Sup/** METH	Immunohistochemistry Methods Development	New experiment methods development.

Project Number	Principal/ Co-Principal Investigator(s)	Status/ Res. Area/ GOAL	Title	Objective
S00032	**Gaylor** Freni Kodell	Active/ **Biometry/** CNPT	Interagency Projects	To participate on interagency committees and workshops, e.g., EPA, DHHS, NIEHS, and NSTC, concerning risk assessment issues.
S00064	**Campbell**	Active/ **Micro/** RSUP	Microbiology Division-media Preparation	To provide microbiological media.
S00069	**Freeman** Deck Evans	Active/ **Chemistry/** RSUP	Equipment Calibration	"Experiment Calibration": routine calibration of all instruments in the division.
S00116	**Gaylor** Chen Freni Kodell	Active/ **Biometry/** CNPT	Risk Assessment (General) (CFSAN) (CDRH) (CVM)	Efforts in the improvement of Risk Assessment.
S00136	**Duffy**	Completed/ **Cal Res/** RSUP	Peromyscus Leucopus Breeding Colony	Maintain breeding stock of Peromyscus leucopus mice to produce experimental animals for future toxicological and nutritional research efforts. A small nucleus of mice from various age groups (1-6 years) will be maintained for life-table studies and to support future research efforts.
S00137	**Hansen**	Active/ **R&D Tox/** RSUP	CD-1 Mouse Breeding Colony	This colony permits the continued maintenance of these mice resulting in large monetary savings over ordering the mice from outside sources. It also facilitates tracking of animal usage and ensures adherence to AALAC/IACUC guidelines.
S00138	**Paule**	Active/ **Neuro Tox/** METH	Nonhuman Primate Operant Behavior Training and Maintenance	To produce and maintain trained animals in NCTR's Operant Test Battery. Animals are primarily Rhesus monkeys. No experimental manipulations such as drug exposure will occur in any subjects under this project number.

Project Number	Principal/ Co-Principal Investigator(s)	Status/ Res. Area/ GOAL	Title	Objective
S00143	**Duffy** Ali Feuers	Active/ **Cal Res/** METH	Development of an Automated Repetitive Blood Sampling System to Measure Circadian Rhythms Of Blood Chemistries	The mechanism of CR action appears to involve effects on the endocrine system. Measurement of these effects is complicated by investigator-generated disruption of animal homeostasis. The system will provide automatic sampling of true levels of sensitive hormonal parameters.
S00158	**Thompson** Lay Baldwin Heinze	Active/ **Chemistry/** METH	FDA Scientific Exchange for Methods Development (CFSAN) (ORA)	The FDA Scientific Exchange for Methods Development program has two related purposes: 1. The interchange of personnel from geographically separated sites builds bridges between people that facilitate the exchange of ideas both with respect to the nature of FDA's analytical chemistry research needs and the solution to outstanding FDA research problems. 2. To promote travel between NCTR and other FDA laboratories that provides scientists access to specialized equipment (or training) that is not available (or cost effective) on-site.
S00162	**Laborde**	Active/ **R&D Tox/** METH	Teratology Training	Train researchers in the techniques of teratological evaluation of rat and/or mouse fetuses.
S00163	**Hansen**	Active/ **R&D Tox/** PRED	Embryo Culture Training	Train researchers in the technique of rodent whole embryo culture using mouse and/or rat fetuses.
S00169	**Ferguson**	Completed/ **R&D Tox/** PRED	Rodent Behavioral Training in Various Tasks	To train rats in the operant behavioral apparatus for later use in other studies and to determine the best method of "shaping" the rat responses.
S00170	**Rafii**	Active/ **Micro/** RSUP	Animals for Antibody Production	To provide antibodies for microbiology program research efforts.

Project Number	Principal/ Co-Principal Investigator(s)	Status/ Res. Area/ GOAL	Title	Objective
S00173	**Gillam**	Active/ **Neuro Tox/** METH	Procedure for Ambulation Exercise of Nonhuman Primates Using the Controlled Ambulation Device (CAD)	To provide training of nonhuman primates in the CAD apparatus.
S00174	**Kodell** Chen Collins Freni Alderson Jacobson Pohland	Active/ **Biometry/** METH	Modification and Application of Quantitative Risk Assessment Techniques to FDA Regulated Products (CDER) (CDRH) (CFSAN) (CVM)	In response to requests from scientists and regulators at CDRH, CDER, CFSAN, and CVM, using available toxicological data, conduct cancer and noncancer risk assessments of FDA regulated products to assist in establishing "safe" conditions of exposure to toxic substance.
S00175	**Kodell** Chen	Active/ **Biometry/** CNPT	Application of Biometrical Procedures for NTP Projects	In response to requests from NCTR scientists, modify and/or apply statistical techniques to the design, conduct, analysis, and interpretation of NTP studies to identify and assess the cancer and noncancer risks of potentially toxic substances.
S00188	**Chen**	Active/ **Biometry/** RSUP	Research Scientists Council	An advisiory committee to the Director and Associate Director for Researhc on issues pertaining to the conduct of scientific research at NCTR.
S00189	**Holland**	Active/ **Micro/** METH	Tuberculocidal Efficiency of Various Disinfectants (CDRH) (ORA)	Assess, modify and validate the AOAC tuberculocidal test procedures for use with disinfectants.
X00006	**Leakey** Hart Seng	Proposed/ **Cal Res/** CNPT	Hypothalmic-Pituitary-Adrenal Axis/Hepatic Rats	To understand the role pancreatic polypeptide, corticosterone, transcortin, CRF, leptin, insulin, eicosanoids and lipocortins play in mediating the effects of caloric restriction in rats.
X00128	**Lyn-Cook** Ellwood Hart Hass Hathcock	Proposed/ **Mol Epi/** CNPT	Zinc Deficiency Pancreatic Acinar Cell *In Vitro*	To examine the effect of marginal zinc deficiency on transformation and to examine the molecular mechanism involved.

Project Number	Principal/ Co-Principal Investigator(s)	Status/ Res. Area/ GOAL	Title	Objective
X30029	**Slikker** Gaylor Sobotka	Proposed/ **Neuro Tox/** METH	Risk Assessment of Neuro-toxicants	To develop and validate quantitative, biologically-based risk assessment procedures for neuro-toxicants.
X40056	**Lyn-Cook** Blann Hart Lyn-Cook	Proposed/ **Mol Epi/** CNPT	Modulation of the MDR Gene by Caloric Restriction	To examine dietary modulation (CR) on the multidrug gene in relation to various chemicals and carcinogenesis.
X40057	**Turturro**	Proposed/ **Cal Res/** CNPT	Effects of Food Restriction on Risk Assessment	Construct risk assessment models which are able to estimate the impact of caloric restriction on spontaneous and induced carcino-genesis.
X50010	**Ali** Duhart Hussain Lipe Meng Newport Schmued Slikker	Proposed/ **Neuro Tox/** AGNT	Iron Induced Oxidative Stress	To determine the role of dietary iron on the occurence of oxidative stress in the rodent central nervous system.
X60001	**Poirier** Cooney Cronin Lopatina Vanyushin	Proposed/ **Mol Epi/** CNPT	Methyl Insufficiency in Carcinogenesis	Project under development.
X60008	**Jackson** Weis	Proposed/ **R&D Tox/** PRED	An *In Vitro* surrogate Assay for Agents Modulating Mammary Cancer	Project under development.
X60010	**Thompson** Allen	Proposed/ **Chemistry/** PRED	Metal Speciation in Foods and Food Packaging	Project under development.
X60016	**Wang** Cerniglia Hairston Henderson	Proposed/ **Micro/** PRED	Effect of Low levels of Antimicrobial Residues in Food on the Human Intestinal Microflora	Project under development.
X60017	**Gehring** Churchwell Doerge Thompson	Proposed/ **Chemistry/** METH	Multiresidue Determination of Sulfonamides in Fish	Project under development.

Project Number	Principal/ Co-Principal Investigator(s)	Status/ Res. Area/ GOAL	Title	Objective
X60021	**Shaddock** Casciano Harris Manjanatha Pipkin	Proposed/ **Gen Tox/** METH	A Study of Several Hepatocarcinogens in the Rat Hepatocyte System: The Effect of AFB1, 2-AAF, Clofibrate and Methapyrilene on Gene Expression and Induction of Stress Proteins	Project under development.
X60027	**Ang** Luo	Proposed/ **Chemistry/** METH	Development of Rapid Methods for Analysis of Vitamins in Feed Products	Project under development.
X60030	**Fu** Ping	Proposed/ **Bio Tox/** CNPT	Effect of Caloric Restriction on Metabolism and DNA Binding of Nitropolycyclic Aromatic Hydrocarbons	Project under development.
X60031	**Beland**	Proposed/ **Bio Tox/** PRED	O-Acetylation of Carcinogenic Hydroxyarylamines	Project under development.
X00032	**Culp**	Proposed/ **Bio Tox** AGNT	2-Year Bioassay on Malachite Green	Project under development
X60033	**Evans**	Proposed/ **Bio Tox/** METH	NMR of Antidepressant Drugs	Project under development.
X60035	**Beland**	Proposed/ **Bio Tox/** AGNT	DNA Adducts from Tamoxifen	Project under development.
X60036	**Roberts** Newkirk	Proposed/ **Bio Tox/** METH	Immunoaffinity Analysis of Oxidative Damage	Project under development.
X60038	**Kadlubar** Ambrosone Huber Tang	Proposed/ **Mol Epi/** PRED	Ovarian Cancer: Bioactivation and DNA Adducts	Project under development.
X60039	**Tang** Lin	Proposed/ **Mol Epi/** PRED	Role of Human CYP1B1 in Carcinogen/Drug Metabolism	Project under development.
X60042	**Paule** Blake	Proposed/ **Neuro Tox/** PRED	Clinical Use of the NCTR Operant Test Battery (OTB)	Project will involve an empirical investigation of OTB performance by normal children and children identified as expressing specific clinical diagnoses including Attention Deficit Disorder With or Without Hyperactivity.

Project Number	Principal/ Co-Principal Investigator(s)	Status/ Res. Area/ GOAL	Title	Objective
X60043	**Rowland** Paule	Proposed/ **Neuro Tox/** CNPT	Risk Factors for Attention Deficit Hyperactivity Disorder (ADHD)	This project will involve an epidemiologic study of several possible environmental risk factors associated with the occurrence of ADHD in a large population of school age children (grades 1-5). Components of the NCTR Operant Test Battery will be used to assist in the clinical assessment of ADHD status.
X60044	**Schmued**	Proposed/ **Neuro Tox/** PRED	Development and Validation of a Neurohistochemical Test Battery	Project under development.
X60048	**Paule** Morris	Proposed/ **Neuro Tox/** AGNT	Grant Proposal to NIDA	Project under development.
X70002	**Hansen** Streck	Proposed/ **R&D Tox/** PRED	Antisense Oligonucleotides	Project under development.
X70004	**Streck** Webb Young	Proposed/ **R&D Tox/** CNPT	Retinoic Acid Receptor Expression	Project under development.
X70005	**Holson** Ferguson	Proposed/ **R&D Tox/** KNLG	Neonatal Glucocoritcoid Exposure	Project under development.
X70006	**Fu**	Proposed/ **Bio Tox/** METH	Study of Secondary Mechanisms: Lipid Peroxidation and Endogenous DNA Adducts Involved in Chloral Hydrate, Benzodiazepines, and Antihistamine Drugs (Newborn Mouse Program)	Project under development.
X70007	**Fu**	Proposed/ **Bio Tox/** METH	Oncogene Analysis of PAH and Nitro PAH Tumors (Newborn Mouse Program)	Project under development.
X70008	**Fu**	Proposed/ **Bio Tox/** METH	Studies on the Effects of Caloric Restriction on Chemically Induced Lipid Peroxidation and DNA Adduct Formation and H-ras Oncogene Activation in Tumor Tissues (Newborn Mouse Program)	Project under development.

Project Number	Principal/ Co-Principal Investigator(s)	Status/ Res. Area/ GOAL	Title	Objective
X70009	**Fu**	Proposed/ **Bio Tox/** METH	EPA-IAG	Project under development.
X70013	**Tolleson**	Proposed/ **Bio Tox/** METH	Spingolipids and Vascular Damage	Project under development.
X70014	**Tolleson**	Proposed/ **Bio Tox/** METH	Age Related Predisposition to Carcinogens	Project under development.
X70016	**Ambrosone** Kadlubar Carino	Proposed/ **Mol Epi/** METH	Determinants of Indolent and Invasive Prostate Cancer	Project under development.
X70017	**Ambrosone** Carino	Proposed/ **Mol Epi/** METH	A Study of Breast Cancer in African-American Women: Innovative Recruitment and Novel Genetic and Environmental Risk Factors	Project under development.
X70020	**Poirier** Cooney Lopatina Wise	Proposed/ **Mol Epi/** PRED	Abnormal DNA Methylation in Hepatocarcinogenesis	Project under development.
X70021	**Poirier**	Proposed/ **Mol Epi/** PRED	Predictive Assays in Hepatocarcinogenesis	Project under development.
X70026	**Manjanatha**	Proposed/ **Gen Tox/** PRED	A Study of DNA Repair in the Transgene of Big Blue Rats	Project under development.
X70029	**Morris** Domon McGarrity	Proposed/ **Gen Tox/** AGNT	Molecular Analysis of Genistein Mutants	Project under development.

Project Number	Principal/ Co-Principal Investigator(s)	Status/ Res. Area/ GOAL	Title	Objective
X70032	**Khan** Cerniglia Khambaty	Proposed/ **Micro/** METH	DNA Finger Printing of Environmental and Clinical P. aeruginosa by PFGE	Pseudomonas aeruginosa has been the most difficult rodent pathogen to control at NCTR. Recently a rapid detection method for identifying P. Aeruginosa form water and food samples has been developed. The pulse field gel electrophoresis (PFGE) analysis of these NCTR isolates and clinical isolates will be performed using rare cut restriction enzymes to establish a database and to determine how these strains are distinct from clinical strains. PFGE will be used to determine if these environmental strains are of the same origin or if they are genetically different thus allowing tracking of the source(s) of contamination.
X70033	**Shuttleworth** Cerniglia Hansen	Proposed/ **Micro/** PRED	Effects of Physio-chemical Factors on the Metabolic Potential of the Drug Metabolizing Fungus, Cunninghamella elegans	The objectives of this study are to determine how various physico-chemical factors affect the drug metabolizing capacity of the fungus Cunninghamella elegans. This fungus has been used as a microbial model for the eukaryotic metabolism of various drugs of pharmacological interest; however, the interrelationship between general fungal physiology and drug metabolism has not been investigated. This study will provide a better understanding of that interrelationship so that we can enhance the production of drug metabolites of interest.0
X70034	**Siitonen**	Proposed/ **Chemistry/** CNPT	ICP/MS and Speciation Analysis of Mn & Cr	Project under development.
X70035	**Doerge** Churchwell Holder	Proposed/ **Chemistry/** METH	LC/MS Determination of Fluroquinolone in Animal Hair	Project under development.
X70036	**Holder** Churchwell Doerge	Proposed **Chemistry/** METH	LC/MS Quantification of Heterocyclic Amines in Meat	Project under development.

Project Number	Principal/ Co-Principal Investigator(s)	Status/ Res. Area/ GOAL	Title	Objective
X70037	**Rushing** Gehring Miller Thompson	Proposed/ **Chemistry/** METH	Uptake and Depletion of Gentian Violet in Catfish	Project under development.
X70039	**Miller** Thompson	Proposed/ **Chemistry/** METH	Waste Disposal Methods for Chemsyn Contract Close Out	Project under development.
X70040	**Lay** Heinze Holland Rafii Sutherland Wilkes	Proposed/ **Chemistry/** KNLG	Bacterial ID Using MALDI-TOF-MS	Project under development.
X70041	**Lay** Heinze Holland Rafii Sutherland Wilkes	Proposed/ **Chemistry/** KNLG	Identification of Bacterial Toxins by LC/MS	Project under development.
X70042	**Pothuluri** Assaf Bloom Cerniglia Haley Nawaz	Proposed/ **Micro/** METH	Microbial Degradation and Fate of Oxytetracycline Used in Aquaculture	Determine the fate and biodegradation of oxytetracycline using radiolabeled compound in a microcosm test system under both oxic and anoxic conditions. Kinetics of sorption/desorption of oxytetracycline will be studied and bacterial resistance in aquaculture samples will be determined.
X70044	**Kodell** Doerge	Proposed/ **Biometry/** METH	Statistical Evaluation of Mass Spec Confirmation Methods for Regulation Purposes	Project under development.
X70045	**Kodell** George	Proposed/ **Biometry/** METH	Trend Test for Clustered Exchangeable Binary Data	Project under development.
X70046	**Arani** Chen	Proposed/ **Biometry/** METH	Adjusted P-values for Multiple Endpoints	Project under development.
X70047	**Wang** Cao Cerniglia Khan	Proposed/ **Micro/** CNPT	Preservation of Foods Using Bacteriocin or Bacteriocin Producing Bacteria: Increasing the Yield of Bacteriocin by Molecular Techniques	Project under development.

Project Number	Principal/ Co-Principal Investigator(s)	Status/ Res. Area/ GOAL	Title	Objective
X70048	**Kodell** Chen Gaylor Zheng	Proposed/ **Biometry/** KNLG	A New Strategy for Detecting Carcinogenicity	Project under development.
X70049	**Sutherland** Castlebury Cerniglia Freeman Holcomb Williams	Proposed/ **Micro/** AGNT	Methods for Detection of Beauvericin and Moniliformin	Development of HPLC and capillary electrophoresis methods for detection of the Fusarium mycotoxins, beauvericin and moniliformin in foods.
X70050	**Sutherland** Cerniglia Lay	Proposed/ **Micro/** METH	Rapid and Sensitive Detection of Staphylococcus Enterotoxin Using Mass Spectrometry Techniques	Project under development.
X70051	**Rafii** Shah	Proposed/ **Micro/** KNLG	Mechanism of Acid Tolerance in Foodborne Pathogens	Project under development.
X70053	**Turturro**	Proposed/ **Cal Res/** AGNT	Model Toxic Response Using Neural Networks	Project under development.
X70055	**LaBorde** Hansen	Proposed/ **R&D Tox/** AGNT	Endocrine Disruptors - Teratology Study	Project under development.
X70059	**Lu**	Proposed/ **Cal Res/** AGNT	Investigation of Reproductive and Developmental Toxicity of Tamoxifen by Flow Cytometric Cell Cycle Analysis	Project under development.

INTERAGENCY AGREEMENTS

INTERAGENCY AGREEMENTS

Cooperating Organization: Environmental Protection Agency - Office of Toxic Substances

Project Number	**Principal**/ Co-Principal **Investigator(s)**	Status/ **Res. Area/** GOAL	Title	Objective
E0663807	**Laborde** Dial Hansen Harmon Holson Jones Leakey Sheehan Webb	Active/ **R&D Tox/** CNPT	Biologically-Based Dose Response Models for Developmental Toxicity: Effect of Dexamethasone (DEX) During Mid- & Late Gestation on CD Rat Dams & on Fetal Amniotic Fluid, Liver Glycogen Concentration and Enzyme Activity	1. To determine the effect of DEX exposure at two gestational periods (GD 9-14 or GD 14-19) on content and volume of amniotic fluid, and biochemistry of liver. 2. To determine the effect of late DEX exposure (GD 14-19) on maternal urine and blood chemistry. 3. To correlate DEX effects on amniotic fluid and on liver function with the stunting, clefting and wavy ribs produced in rat fetuses by this drug. 4. To assess the degree to which DEX-induced anorexia contributes to any of the above effects
E0667800	**Fu** Dooley Kadlubar	Active/ **Bio Tox/** PRED	Neonatal Mouse Bioassay of Eight Complex Mixtures and Three Positive Control Samples	1. To use neonatal male B6C3F1 mice to determine the tumorigenic activity of eight complex mixtures samples (i.e., smoky coal, aluminum smelter emissions, ambient air, cigarette smoke, coke oven emissions, diesel exhaust, polyethylene incineration and roofing tar), three positive control samples (i.e., benzo(a)pyrene, 6-nitrochrysene, and 4-aminobiphenyl) and their carrier (DMSO). 2. To remove target tissues from treated animals and send to EPA for carcinogen-DNA adduct quantitation and characterization. 3. To prepare appropriate synthetic standards for carcinogen-DNA adduct detection.
E0667820	**Fu** Dooley Kadlubar	Active/ **Bio Tox/** AGNT	Mouse Skin Tumor Initiating Activity of Two Ambient Air Samples	Use female Sencar mice to determine the skin tumor initiating activity of two organic extracts of ambient air particulate matter, provided by EPA.

Cooperating Organization: National Institute on Aging

Project Number	**Principal/** Co-Principal **Investigator(s)**	Status/ **Res. Area/** GOAL	Title	Objective
E0049400	**Turturro** Hart	Active/ **Cal Res/** CNPT	Caloric Restriction in Fischer 344 Rats	Breed and age Fischer 344 calorically restricted rats.
E0050100 thru E0050805	**Turturro**	Active/ **Cal Res/** CNPT	NIA-IAG Caloric Restriction and Aging	Breed and age calorically restricted rodents.
E0050900	**Turturro** Gaylor Hart Sheldon	Active/ **Cal Res/** CNPT	F-344 Rat Fed Ralston-Purina Masoro Mod. Diet	Selected diet for caloric restriction.
P00304	**Turturro**	Active/ **Cal Res/** AGNT	NIA IAG Administrative Overhead	General administration of PCR IAG.

Cooperating Organization: National Institute of Drug Abuse

Project Number	**Principal/** Co-Principal **Investigator(s)**	Status/ **Res. Area/** GOAL	Title	Objective
E0663306	**Paule** Binienda	Active/ **Neuro Tox/** AGNT	ADDEND: Preliminary Studies for Determining the Effects of Chronic Cocaine Exposure during Pregnancy on the Behavior of Offspring in Monkeys	Increase the number of offspring in the total gestational exposure (TGE) group to ten. Requesting that 10 nonpregnant animals be maintained under chronic cocaine treatment while they are in the breeding program until at least 10 viable offspring are available. Requesting another 7 animals for inclusion in control group to bring the total to 10.

Cooperating Organization: National Institute of Environmental Health Sciences

Project Number	**Principal**/ Co-Principal **Investigator(s)**	Status/ **Res. Area/** GOAL	Title	Objective
E0250001 E0250011	**Schmued** **Sandberg** Appel Blann Bo Lyn-Cook Paule Schmued Slikker	Active/ **Neuro Tox/** METH	Preliminary Assessment of a Method for Screening the Potential Neurotoxic Effects of Anti-HIV Therapeutics Using Autoradiographic Measurement of Cellular Metabolic Markers (CBER)(CDER)	To examine, validate and compare the utility of a number of biological markers (2'3'-d-dideoxycytidine (ddC), 2,3'-d-dideoxyinosine (ddI), isoniazid) of neurotoxic insults using *in vivo* and *in vitro* autoradiographic methods.
E0250101	**Patterson** Binienda Slikker Sandberg Lipe Gillam	Active/ **Neuro Tox/** AGNT	Placental Transfer and Fetal Distribution of the Human Immunodeficiency Virus (HIV) Therapeutics: 3'-azido-2',3-dideoxythymidine (AZT), 2'3'-dideoxylinosine (ddI), and 2',3'-didehydro-2'3'-dideoxythymidine (d4T) (CDER)	To determine the placental and fetal distribution of AZT, ddI and d4T, and their phosphorylated metabolites in the later-term rhesus monkey.
E0250201	**Patterson** Paule Sandberg Schmued Sheevers Slikker Zielinski	Active/ **Neuro Tox/** PRED	Neurotoxicological and Behavioral Assessment of the Human Immunodeficiency Virus (HIV) Suppressors 2',3'-dideoxycytidine (ddC) and Thalidomide in Rhesus Monkeys (CDER)	To assess the neurotoxicity and neurobehavioral effects of chronic treatment with the anti-HIV agents 2',3'-dideoxycytidine (ddC) and thalidomide in rhesus monkeys.
E0672504	**Schmued** Ferguson Morse Paule Slikker	Complete/ **Neuro Tox/** PRED	Use of Rodent Operant Test Battery (OTB) and Other Tests to Assess Neurobehavioral Toxicity of ddC (CDER)	To assess the effects of the anti-HIV therapeutic ddc treatment on rodent operant and other behaviors and to compare the results obtained with those of a similar study conducted in monkeys using the NCTR OTB (E0672500).
E0672570 E0672571 E0672527 E0672537	**Sandberg** **Lyn-Cook** Binienda Blann Bo Nickols Paule Schrader Slikker Taylor	Complete/ **Neuro Tox** **&** **Nutri Tox/** PRED & CNPT	Neurotoxicity Assessment of Anti-Human Immunodeficiency Virus (HIV) Therapeutics in Nonhuman Primates: Comparison of ddI to Isoniazid	To determine if ddI exerts any molecular changes, such as, mutations, activation of oncogenes and epigenetic effect on the DNA in pancreatic tissue or pancreatic acinar cells. Pathological changes observed in these tissues will be correlated with molecular changes noted.

Cooperating Organization: National Toxicology Program

Project Number	**Principal/** Co-Principal **Investigator(s)**	Status/ **Res. Area/** GOAL	Title	Objective
E0210001	**Fu** Casciano Heinze Kadlubar Von Tungeln Contrera	Active/ **Bio Tox/** AGNT	Metabolism and DNA Binding of Chloral Hydrate (CDER)	1. Characterize and quantify the metabolites of chloral hydrate 2. Determine the mechanism of metabolic activation of chloral hydrate 3. Prepare synthetically carcinogen modified DNA adduct(s) of chloral hydrate and its metabolites 4. Determine the principal metabolizing responsible for metabolic activation 5. Study mutagenicity, metabolism and DNA adduct formation of chloral hydrate and its metabolites
E0210101	**Beland** Contrera Dooley	Active/ **Bio Tox/** AGNT	Fourteen-day, Repeat-dose, Range-finding Study of Chloral Hydrate in Male and Female B6C3F1 Mice (CDER)	To determine the doses of chloral hydrate to be used in a chronic study.
E0210201	**Beland** Contrera Dooley	Active/ **Bio Tox/** AGNT	Fourteen-Day, Repeat-Dose, Metabolism Study of Chloral Hydrate in Male and Female B6C3F1 Mice (CDER)	To establish the plasma levels of chloral hydrate and its metabolites in B6C3F1 mice.
E0210301 E0210311	**Beland** Contrera Dooley	Active/ **Bio Tox/** AGNT	Fourteen-Day, Repeat-Dose, Range-Finding Study of Chloral Hydrate in Male and Female Fischer 344 (F344) Rats (CDER)	To determine the doses of chloral hydrate to be used in a chronic study.
E0210401 E0210411	**Beland** Contrera Dooley	Active/ **Bio Tox/** AGNT	Fourteen-Day, Repeat-Dose, Metabolism Study of Chloral Hydrate in Male and Female Fischer 344 (F344) Rats (CDER)	To establish the plasma levels of chloral hydrate and its metabolites in Fischer 344 Rats.
E0210601	**Howard** Dooley Lorentzen Voss	Active/ **Bio Tox/** AGNT	Chronic Tumor Study of Fumonisin B1 in Male and Female B6C3F1 Mice (CFSAN)	To determine the tumorigenicity of fumonisin B1 in male and female B6C3F1 mice following chronic dietary exposure.
E0210801	**Howard** Dooley Lorentzen Voss	Active/ **Bio Tox/** AGNT	Chronic Tumor Study of Fumonisin B1 in Male and Female F344 Rats (CFSAN)	To determine the tumorigenicity of fumonisin B1 in male and female F344 rats following chronic dietary exposure.

Cooperating Organization: National Toxicology Program

Project Number	**Principal/** Co-Principal **Investigator(s)**	Status/ **Res. Area/** GOAL	Title	Objective
E0211001 E0211011 E0211012 E0211013	**Laborde** Bucci Collins Flynn Hansen Howard Shackleford Terry	Active/ **R&D Tox, Bio Tox & Path/** AGNT	Developmental Toxicity Study of Fumonisin B_1 in Rabbits (CFSAN)	To determine the effect of Fumonisin B1 on rabbit development by administering the compound during organogenesis.
E0211101 E0211111 E0211121 E0211131 E0211141	**Howard** Binienda Casciano Couch Martinez Melchior Shaddock Slikker Sutherland Tolleson	Active/ **Bio Tox/** AGNT	The Role of Fumonisin B_1 in Fusarium sp. Tumorigenicity in Rats (CVM)	Determine the effect of fumonisin B1 on signal transduction pathways in cultured human esophageal epithelial tissues. Determine if DNA damage occurs *in vivo* in F344 rats when fed in the diet cultures of Fusarium graminearum, Fusarium subglutinans, Fusarium moniliforme or a combination of the three fungi, using 32P-postlabeling technique. Determine the pharmacokinetics of fumonisin B1 in B6C3F1 mice and F344 rats under conditions similar to those used in the chronic bioassay, and in non-human primates.
E0211201	**Howard** Dooley	Active/ **Bio Tox/** AGNT	Tumor Promotion by Fumonisin B1 in Male F344 Rats (CFSAN)	Determine if fumonisin B1 is a tumor promoter, using a classical initiation/ promoter design [administration of fumonisin B1 one week after three weeks administration of methylbenzylnitrosamine]. Determine if co-administration of fumonisin B1 with initiator methylbenzylnitrosamine results in an altered tumor yield.
E0211301 E0211311 E0211321	**Howard** Dooley Lorentzen Voss	Active/ **Bio Tox/** AGNT	Sub-chronic (28-day) Study of Fumonisin B1 in Male and Female B6C3F1 Mice (CFSAN)	To determine the toxicity of fumonisin B1 in male and female B6C3F1 mice following a 28-day dietary exposure.
E0211401 E0211411	**Howard** Dooley Lorentzen Voss	Active/ **Bio Tox/** AGNT	Sub-chronic (28-day) Study of Fumonisin B1 in Male and Female F344 Rats (CFSAN)	To determine the toxicity of fumonisin B1 in male and female F344 rats following a 28-day dietary exposure.

Cooperating Organization: National Toxicology Program

Project Number	**Principal**/ Co-Principal **Investigator(s)**	Status/ **Res. Area/** GOAL	Title	Objective
E0211511	**Slikker** Sobotka	Complete/ **Neuro Tox/** AGNT	ADDEND: Developmental Neurotoxicological Assessment of Fumonisin (FB_1) Toxicosis in Rats (CFSAN)	To assess behavioral and growth effects in postnatal rats after prenatal exposure to purified FB1. Addendum requested in order to replicate findings from E06884.01 and provide data from a higher dose that would show maternal toxicity.
E0211601	**Beland** Contrera Fullerton Gaylor	Active/ **Bio Tox/** AGNT	Tumorigenicity of Chloral Hydrate in B6C3F1 Mice (CDER)	To determine the effect of animal age and duration of exposure upon the tumorigenicity of chloral hydrate in female B6C3F1 mice.
E0211701 X70052	**Leakey** Turturro Seng Contrera	Active/ **Cal Res/** CNPT	Chronic Bioassay of Chloral Hydrate in Male B6C3F1 Mice Using Idealized Body Weight Curves that are Normalized by Modulation of Caloric Intake (CDER)	To determine the chronic toxicity and potential carcinogenicity of chloral hydrate, administered by aqueous gavage, to male B6C3F1 mice; To determine the feasibility of utilizing dietary control (i.e., the manipulation of caloric intake) to control body weight gain so that all mice in each experimental group of the bioassay conform to an ideal weight curve.
E0211801	**Culp** Mulligan Beland	Active/ **Bio Tox/** AGNT	Twenty-eight Day Range finding Study in Mice and Rats Administered Malachite green or Leucomalachite Green in the Diet (CVM)	To determine the doses of malachite green to be used in a two-year feeding bioassay and to compare the biological effects from the administration of malachite green and leucomalachite green.
E0211901 E0211911	**Doerge** Rushing Churchwell Schmitt	Active/ **Chemistry/** METH	Development of Analytical Methods for Determination of Malachite Green	1) Develop analytical methods to assess purity of malachite green (MG) and leuco-malachite green (LMG) that will be used in the NTP animal bioassay; 2) Develop analytical methods to quantify MG and LMG content and determine homogeneity and stability in rodent chow under storage and use condition.
E0212001 P00378	**Beland** Benson Chan Lorentzen Roberts	Active/ **Bio Tox**/ AGNT	Effect of Ethanol on the Tumorigenicity of Urethane (Ethyl Carbamate) in B6C3F1 Mice (CFSAN)	To determine the effect of ethanol on the tumorigenicity of urethane (ethyl carbamate) in B6C3F1 mice.

Cooperating Organization: National Toxicology Program

Project Number	**Principal/** Co-Principal **Investigator(s)**	Status/ **Res. Area/** GOAL	Title	Objective
E0212101 P00367	**Doerge** Syvertson	Active/ **Chemistry/** AGNT	Development of Analytical Methods for Determination of Urethane (CFSAN)	Develop analytical methods to assess purity and stability of urethane and ethanol that will be used as test compounds in the NTP rodent bioassay; Develop analytical methods to quantify urethane and ethanol content in aqueous dosing solutions and determine stability under storage and use conditions for the NTP bioassay; Develop analytical procedures to quantify the content of urethane in rodent feed.
E0212201 P00373	**Delclos** Newbold Weis	In Review/ **Bio Tox/** AGNT	Range Finding Study for the Evaluation of the Toxicity of Genistein Administered in the Feed to CD (Sprague-Dawley) Rats	To determine the doses of genistein to be used in a multigeneration bioassay for establishing the effects of this naturally occurring isoflavone on development of reproductive organs, reproduction, cancer of the reproductive organs, and neurological and immunological function.
E0212301 P00372	**Delclos** Newbold Weis	In Review/ **Bio Tox/** AGNT	Range Finding Study for the Evaluation of the Toxicity of Methoxychlor Administered Feed to CD (Sprague-Dawley) Rats	To determine the doses of methoxychlor for use in a multigeneration bioassay for assessing the effects of this pesticide on the development of the reproductive tract, reproduction, cancer of the reproductive organs, and neurological and immunological function.
E0694101	**Roberts** Benson Howard Newkirk Tolleson	Active/ **Bio Tox/** AGNT	Preparation of Antibodies Against the C1-C10 and Tricarballylic C14-C20 Segments of Fumonisin B1: Development for Quantitative and Molecular Biological Techniques	1) Prepare fumonisin B1-protein conjugates for immunization, immunoassay development, and epitope mapping; 2) raise polyclonal anti-fumonisin B1 adduct antisera and characterize titer, affinity, and relevant cross reactivity; 3) evaluate the usefulness of anti-fumonisin B2 antisera to elucidate target organ toxicity and as a tool to isolate or localize macromolecules modified by or binding fumonisin B1; 4) prepare immunoaffinity matrices and evaluate immunoaffinity techniques to enrich/concentrate/purify fumonisin B1 as an aid to the identification and quantification of fumonisin B1 in biological samples including food.

Cooperating Organization: National Toxicology Program

Project Number	**Principal**/ Co-Principal **Investigator(s)**	Status/ **Res. Area/** GOAL	Title	Objective
P00293	**Beland** Contrera	Completed/ **Bio Tox/** AGNT	NTP Standard Bioassay (CDER)	This project set up to capture time charges for NTP work or protocol review of NTP-related projects prior to official approval of the project.
P00321	**Thompson** Holcomb Nestorick	Completed/ **Chemistry/** AGNT	NTP Preliminary Investigation on Fumonisin	To provide analytical chemistry support for NTP Fumonisin studies.
P00345	**Culp** Blankenship Beland	Complete/ **Bio Tox/** METH	Development of Methods for Extracting and Analyzing Malachite Green in Rodent Tissues	To develop extraction, HPLC and 32P-postlabeling methods for detecting and analyzing chromatic malachite green, leucomalachite green, malachite green carbinol, and malachite green DNA-adducts in rodents gavaged with malachite green.
P00374	**Delclos**	Active/ **Bio Tox/** AGNT	Preparation of a Comprehensive Research Plan: Synthetic and Naturally Occurring Endocrine Disrupters	Overall research plan for the NTP/NCTR IAG to determine the effects of endocrine disrupting chemicals on fertility and reproductive tract cancers.
X60032	**Culp** Blankenship	Proposed/ **Bio Tox/** AGNT	2-Year Bioassay on Malachite Green	To be developed in FY-97.
X6005101	**Delclos** Beland Hansen Littlefield Sheehan	Proposed/ **Bio Tox/** AGNT	Multi-Generation Studies (Dose Range Finding Study - Compound to be Determined)	To be developed.
X6005102	**Delclos**	Proposed/ **Bio Tox/** AGNT	Multi-Generation Studies (Dose Range Finding Study - Vinclozolin)	To be developed.
X6005103	**Delclos**	Proposed/ **Bio Tox/** AGNT	Multi-Generation Studies (Dose Range Finding Study - Nonylphenol)	To be developed.
X60052	**Delclos**	Proposed/ **Bio Tox/** AGNT	Multi-Generation Studies (OF Generation for Reproductive Assessment - Genistein)	To be developed.
X60053	**Delclos**	Proposed/ **Bio Tox/** AGNT	Multi-Generation Studies (OF Generation for Immuno/Neuro-tox Assessment - Genistein)	To be developed.

Cooperating Organization: National Toxicology Program

Project Number	**Principal/** Co-Principal **Investigator(s)**	Status/ **Res. Area/** GOAL	Title	Objective
X60054	**Delclos**	Proposed/ **Bio Tox/** AGNT	Multi-Generation NTP Studies (OF Generation for Cancer Assessment - Genistein)	To be developed.
X60055	**Delclos**	Proposed/ **Bio Tox/** AGNT	Multi-Generation Studies (F1 Generation for Reproductive Assessment - Genistein)	To be developed.
X60056	**Delclos**	Proposed/ **Bio Tox/** AGNT	Multi-Generation Studies (F1 Generation for Cancer Assessment - Genistein)	To be developed.
X60057	**Delclos**	Proposed/ **Bio Tox/** AGNT	Multi-Generation Studies (F2 Generation for Reproductive Assessment - Genistein)	To be developed.
X60060	**Sheehan** Branham Vom Saal Welshons	Proposed/ **R&D Tox/** AGNT	Dose Selection Strategy for Estrogenic Endocrine Disruptors - Endocrine Disrupters - Uterine Tissue	To be developed.
X70010	**Howard**	Proposed/ **Bio Tox/** METH	Isolation of Human Genomic Ceramide Synthetase	To be developed.
X70011	**Howard**	Proposed/ **Bio Tox/** METH	DNA Adducts from the Pyrrolizidine Alkaloid Riddelline	To be developed.
X70012	**Howard**	Proposed/ **Bio Tox/** METH	Comparative Toxicity of Fumonisins in Rats	To be developed.

COOPERATIVE RESEARCH AND DEVELOPMENT AGREEMENTS

Cooperating Organization: American Institute for Cancer Research

Project Number	**Principal/** Co-Principal **Investigator(s)**	Status/ **Res. Area/** GOAL	Title	Objective
E0260101 E0260111 E0260112	**Djuric**[********] **Hart** Lewis Lu	Active/ **Cal Res/** CNPT	Modulation of Oxidative DNA Damage in Rats by Diet	1. To examine the relationships between the level of oxidative DNA damage and fat intake. 2. To examine the relationship between the level of oxidative DNA damage and energy intake.
E0260201 E0260211 E0260221	**Gandy**[*********] Leakey Manjgaladse Seng	Active/ **Cal Res/** CNPT	Effect of Caloric Restriction on Rat Testicular Tumor Formation	All of the aims of this proposal are directed towards understanding the role of dietary components (i.e., caloric restriction) in influencing the ultimate susceptibility of the male reproductive tract to chemical insult.
E0260301 E0260311 E0260313 E0260321 E0260331	**Wolff** Kaput Visek	Active/ **Bio Tox/** CNPT	Caloric Restriction and Gene Expression in Agouti Mice (CDER)	The total amount of fat and calories we consume in our diet is highly correlated with the occurrence of cancer in North America and other highly developed nations. The studies proposed will help us learn how calories modify the development of cancer in mice and the mechanism underlying cancer development in humans.

Cooperating Organization: Electric Power Research Institute

Project Number	**Principal/** Co-Principal **Investigator(s)**	Status/ **Res. Area/** GOAL	Title	Objective
E0672201	**Beland** Culp Dooley	Active/ **Bio Tox/** CNPT	Twenty-One Day Diet Range-finding Study	1. Develop methods for mixing coal tar residues in NIH-31 diets at various concentrations. 2. Determine the palatability of a representative coal tar mixture that will be used in a subsequent chronic bioassay. 3. Develop methods to quantify DNA adducts by 32P-postlabeling.

[********]Wayne State University

[*********]University of Arkansas for Medical Sciences

Cooperating Organization: Electric Power Research Institute

Project Number	**Principal**/ Co-Principal **Investigator(s)**	Status/ **Res. Area/** GOAL	Title	Objective
E0672202	**Beland** Culp Dooley Fullerton Kaderlik	Active/ **Bio Tox/** CNPT	Chronic Bioassay of Two Composite Samples From Selected Manufactured Gas Plant Waste Sites	To determine the risk associated with exposure to coal tar mixtures obtained from manufactured gas plant waste sites.
E0672203 E0672223 E0672231 E0672233	**Culp** Beland Dooley	Active/ **Bio Tox/** CNPT	DNA Adduct and Cell Replication Analyses of Mice Treated w/Two Composite Samples from Selected Manufactured Gas Plant Waste Sites	To determine the relationships among tumor induction, DNA adduct formation, and cell replication activity in female B6C3F1 mice resulting from exposure to coal tar mixtures obtained from manufactured gas plant waste sites.
E0675200	**Cerniglia**	Active/ **Micro/** CNPT	The Role of Human Intestinal Microflora in The Metabolism of Compounds in Gas Manufacturing Plant Residues	1. To determine the effect of compounds in gas manufacturing plant residues on the microbial activity and ecology of human intestinal microflora. 2. To determine the role of human intestinal microflora in metabolizing compounds contained in gas manufacturing plant residues.

Cooperating Organization: Procter & Gamble

Project Number	**Principal/** Co-Principal **Investigator(s)**	Status/ **Res. Area/** GOAL	Title	Objective
E0270001	**Jackson** Detilleux	Active/ **R&D Tox/** CNPT	Role of Hepatotoxicity in the Induction of Liver Tumors in B6C3F1 Mice Treated with Doxylamine, Pyrilamine, and Triprolidine (CDER)	The hypothesis to be tested is that liver tumors resulting from administration of doxylamine to mice is an indirect result of hepatotoxicity and cell proliferation induced by doxylamine. The objective is to test the hypothesis by re-examining liver tissues of male mice from previous NCTR studies on doxylamine, triprolidine, and pyrilamine for hepatotoxicity and cell proliferation and to correlate this with liver tumor incidence at each dose level and length of exposure.

Cooperating Organization: Gentest Corporation

X70054	**Leakey** Seng	Proposed/ **Cal Res/** PRED	Predictive ystems for Human Drug Metabolism	Determine whethr age, disease, diet and/or body mass influence expression of hepatic drug metabolizing enzymes in monkeys and humans; To assess what influence altered drug metabolizing enzyme expression with have on drug efficacy and toxicity.

1996 NCTR PUBLICATIONS

1996 NCTR PUBLICATIONS

1. Ahn, H. and Chen, J.J. Tree-structured logistic model for over-dispersed binomial data with application. Biometrics, Accepted: 8/16/96. **(E0685400) (KNOWLEDGE BASE) (Biometry)**

2. Ahn, H. Log-normal regression modeling through recursive partitioning. Computational Statistics and Data Analysis, 21:381-398, 1996. **(KNOWLEDGE BASE) (Biometry)**

3. Alexandrov, K., Rojas, M., Kadlubar, F.F., Lang, N.P., and Bartsch, H. Evidence of anti-benzo[a]pyrene diolepoxide-DNA adducts in human colon mucosa. Carcinogenesis, 17(9):2081-2083, 1996. **(E0694700) (CONCEPT-DRIVEN) (Mol Epi)**

4. Ali, S.F., Duhart, H.M., Newport, G.D., Lipe, G.W., and Slikker, W., Jr. Manganese-induced reactive oxygen species: Comparison between Mn+2 and Mn+3, Neurodegeneration, 4:329-334, 1995. **(E0671900) (AGENT-DRIVEN) (Neuro Tox)**

5. Allen, L.B., Siitonen, P.H., and Thompson, H.C. Aerosol-phase assisted sample digestion for the determination of trace metals in organic samples by plasma atomic emission spectrometry. Journal of Analytical Atomic Spectrometry, 11:529-532, 1996. **(E0698201) (METHOD-DRIVEN) (Chemistry)**

6. Ambrosone, C.B., Freudenheim, J.L., Graham, S., Marshall, J.R. Vena, J.,E., Brasure, J.R., Michalek, A.M., Laughlin, R., and Nemoto, T. Cigarette Smoking, N-acetyltransferase 2 genetic polymorphisms, and breast cancer risk. J. American Medical Association, 276(18):1494-1501, 1996. **(E0697800) (Predictive Toxicity) (Mol Epi)**

7. Andersen, S.L., and Gazzara, R.A. Effect of (-) sulpiride on dopamine release in striatum of developing rats: Degree of depolarization influences responsiveness. J Neurochemistry, Accepted: 6/13/96. **(CONCEPT-DRIVEN) (R&D Tox)**

8. Anderson, K., Hammons, G.J., Ilett, K., Kadlubar, F.F., Potter, J.D., Kaderlik, K., Minchin, R.F., Teitel, C.H., Chou, H., and Guengerich, F.P. Metabolic activation of aromatic amines by human pancreas. Carcinogenesis, Accepted 10/17/96. **(E0694601) (Predictive Toxicity) (Mol Epi)**

9. Ang, C.Y. and Liu, F. Influence of heating end-point temperture on violatiles from poultry meat - A review. Journal of Muscle Foods, 7:291-302, 1996. **(E0693601) (METHOD-DRIVEN) (Chemistry)**

10. Ang, C.Y. and Luo, W. Rapid determination of ampicillin in bovine milk by liquid chromatography with fluorescence detection. Journal of AOAC International, Accepted: 9/23/96. **(E0693601) (METHOD-DRIVEN) (Chemistry)**

11. Ang, C.Y., Liu, F., and Huang, Y.W. Catalyatic activity as an indicator of end-point temperatures in heat processed, commercially prepared chicken patties. Journal of Muscle Foods, 7:345-354, 1996. **(E0693601) (METHOD-DRIVEN) (Chemistry)**

12. Ang, C.Y., Luo, W., Hansen, E., Freeman, J.P., and Thompson, H.C. Determination of amoxicillin in catfish and salmon tissues by liquid chromatography with pre-column formaldehyde derivatization. Journal of AOAC International, 2:389-396, 1996. **(E0693001) (AGENT-DRIVEN) (Chemistry)**

13. Badawi, A.E., Stern, S.J., Lang, N.P., and Kadlubar, F.F. Cytochrome P-450 and acetyltransferase expression as biomarkers of carcinogen-DNA adduct levels and human cancer susceptibility. Progr. Clin. Biol. Res., 395:109-140, 1996. **(CONCEPT-DRIVEN) (Mol Epi)**

14. Basnakian, A.G. and James-Gaylor, S.J. Quantification of 3'OH DNA breaks by random oligonucleotide-primed synthesis DNA and Cell Biology, 15:255-262, 1996. **(CONCEPT-DRIVEN) (Bio Tox)**

15. Basu, A., Gaylor, D.W., and Chen, J.J. Estimating the probability of occurrence of tumor for a rare cancer with zero occurrence in a small sample. Regulatory Toxicology and Pharmacology, 23:139-144, 1996. **(METHOD-DRIVEN) (Biometry)**

16. Beland, F.A., Melchior, W.A., Mourato, L.L.G., Santos, M.A., and Marques, M.M. Arylamine-DNA adduct conformation in relation to mutagenesis. Mutation Research, In Press. **(E0673700) (PREDICTIVE TOXICITY) (Bio Tox)**

17. Bezalel, L., Hadar, Y., Fu, P.P., Freeman, J.P., and Cerniglia, C.E. Initial oxidation products in the metabolism of pyrene, anthracene, and dibenzothiophene by the white-rot fungus *Pleurotus ostreatus*. Applied and Environmental Microbiology, 62(7):2554-2559, 1996. **(CONCEPT-DRIVEN) (Micro)**

18. Bezalel, L., Hadar, Y., Fu, P.P., Freeman, J.P., and Cerniglia, C.E. Metabolism of phenanthrene by the white-rot fungus *Pleurotus ostreatus*. Applied and Environmental Microbiology, 62:2547-2553, 1996. **(CONCEPT-DRIVEN) (Micro)**

19. Billedeau, S.M., Holcomb, M., Liu, X., and Wilkes, J.G. Analysis of fumonisins FB_1, FB_2, and FB_3 using LC-ELSD and LC-FTIR. Journal of Hyphenated Analytical Strategies, 1:237-243, 1996. **(AGENT-DRIVEN) (Chemistry)**

20. Billedeau, S.M., Vollmer, D.L., Wilkes, J.G., and Thompson, H.C. Detection of N-nitrosamines in raw materials used in cosmetic formulations following nitrosation

by LC/ELSD and PB/TEA with EI and ESI/MS confirmation. Real Time Analytical Strategies Using Hyphenated Techniques, Vol. 2, 1996, In Press. **(METHOD-DRIVEN) (Chemistry)**

21. Binienda, Z.K., Holson, R.R., Chen, F., Oriaku, E.T., and Kim, C. Effects of ischemia-hypoxia induced by interruption of uterine blood flow on fetal rat liver and brain enzyme activities and offspring behavior. Int. J. Devl. Neuroscience, 14(4):399-408, 1996. **(E0686901) (CONCEPT-DRIVEN) (Neuro Tox)**

22. Binienda, Z.K., Sandberg, J.A., Slikker, W., Jr., and Ali, S.F. Alterations in electroencephalographic signals and monoamine concentrations in the rat brain following cocaine and methamphetamine treatment. Annals of New York Academy of Sciences, 3-2714:561-573, 1996. **(E0686901) (CONCEPT-DRIVEN) (Neuro Tox)**

23. Bowyer, J.F., Clausing, P.P., Schmued, L.C., Davies, D.L., Binienda, Z.K., Scallet, A.C., and Slikker, W., Jr. Parenterally administered 3-nitropropionic acid and amphetamine can combine to produce damage to terminals and cell bodies in the striatum. Brain Research, 712:221-229, 1996. **(E0690301) (CONCEPT-DRIVEN) (Neuro Tox)**

24. Bucci, T.J. and Howard, P. Effect of fumonisin mycotoxins in animals. Toxin Review, 15(3):293-302, 1996. **(AGENT-DRIVEN) (Research)***

25. Bucci, T.J., Hansen, D.K., and Laborde, J.B. Leukoencephalomalacia and Hemorrhage in the brain of rabbits gavaged with mycotoxin fumonisin B_1. Natural Toxins, 4:51-52, 1996. **(E0211002) (AGENT-DRIVEN) (Research)***

26. Butcher, N.J., Minchin, R.F., Kadlubar, F.F., and Ilett, K. Uptake of the food-derived heterocyclic amine carcinogen 2-amino-1-methyl-6- phenylimidazo(4,5-b)- pyridine (PhIP) and its N-hydroxy metabolite (N-OH-PhIP) into rat pancreatic acini and hepatocytes in vitro. Carcinogenesis, 17:889-892, 1996. **(E0696701) (AGENT-DRIVEN) (Mol Epi)**

27. Cadet, J., Rothman, R., and Ali, S.F. Superoxide radicals mediate the biochemical effects of methylenedioxymethamphetamine (MDMA): evidence from using CuZn-superoxide dismutase transgenic mice. Synapse, Accepted: 6/19/96. **(E0688701) (CONCEPT-DRIVEN) (Neuro Tox)**

28. Capen, C.C., Grone, A., Bucci, T.J., and Rosol, T.J. Age-related changes in structure and function of the mouse parathyroid gland. ILSI Monography, Pathobiology of the Aging Mouse. Accepted: 1/01/96. **(E0050100) (CONCEPT-DRIVEN) (Research)***

29. Carter, C.A., Pogribna, M.V., Davidson, A.D., Jackson, C.D., McGarrity, L.J., and Morris, S.M. Effects of retinoic acid on cell differentiation and reversion toward a normal phenotype in human endometrial adenocarcinoma (RL96-2) cells. Anticancer Research, 16:17-24, 1996. **(E0685200) (AGENT-DRIVEN) (R&D Tox)**

30. Casciano, D.A., Chou, M.W., Lyn-Cook, L.E., and Aidoo, A. Calorie restriction modulates chemically-induced in vivo somatic mutation frequency and perhaps spontaneous mutation frequency. Environmental and Molecular Mutagenesis, 27:162-164, 1996. **(E0676700) (PREDICTION OF TOXICITY) (Gen Tox)**

31. Casillas, R., Heinze, T. M., Deck, J., and Crow, S.A. Initial oxidative and subsequent conjugative metabolites produced during the metabolism of phenanthrene by fungi. Journal of Industrial Microbiology, 16:205-215, 1996. **(METHOD-DRIVEN) (Micro)**

32. Cerniglia, C.E. Assessing the effects of antimicrobial residues in food on the human intestinal microflora. World Health Organization: Report for the FAO/WHO Joint Expert Committee on Food Additives, 1996. **(PREDICTION OF TOXICITY) (Micro)**

33. Chae, Y.-H., Delclos, K.B., and El-Bayoumy, K. Metabolism and DNA binding of the environmental colon carcinogen 6-nitrochrysene in rats. Cancer Research, 56:2052-2058, 1996. **(E0655500) (AGENT-DRIVEN) (Bio Tox)**

34. Chae, Y.-H., Upadhyaya, P., Ji, B.-Y., Fu, P.P., and El-Bayoumy, K. Comparative metabolism and DNA binding of 1-,2-, and 4-nitropyrene in rats. Mutation Research, In Press, 1996. **(E0657300) (PREDICTIVE TOXICITY) (Bio Tox)**

35. Chiu, L.H., Von Tungeln, L.S., Deck, J., Vingiello, F.A., Ueng T.-H., and Fu, P.P. Synthesis and rat liver microsomal metabolism of 2-chlorodibenz[a,l]pyrene and 10-chlorodibenz[a,l]pyrene. Polycyclic Aromatic Compounds, In Press, 1996. **(E0657300) (PREDICTIVE TOXICITY) (Bio Tox)**

36. Chang, H.C., Compadre, L., Lloyd, R.V., Freeman, J.P., and Samokyszyn, V. Nonenzymatic reduction of tetrachloro-1,4-benzoquinone by reduced nicotinamide adenine dinucleotide phosphate in an aqueous system. Biochemical and Biophysical Research Communications, 220 (3):1043-1048, 1996. **(CONCEPT-DRIVEN) (Chemistry)**

37. Chen, J.J. and Ahn, H. Mixed poisson regression models using Quasi-likelihood methods (Mixed Poisson regression models using semi-parametric methods). Biometric Journal, 38:81-96, 1996. **(E0684300) (METHOD-DRIVEN)**

38. Chen, J.J. and Lensing, S.Y. Causal modeling of multivariate outcomes from developmental toxicity experiments. Environmental and Ecological Statistics, 3:207-218, 1996. **(S00009) (CONCEPT-DRIVEN) (Biometry)**

39. Chen, J.J., Gaylor, D.W., and Laborde, J.B. Dose-response modelling of growth for developmental toxicity. Environmentics, 7:135-144, 1996. **(E0663806) (PREDICTIVE TOXICITY)**

40. Chen, J.J. Global tests for analysis of multiple tumor data from animal carcinogenicity experiments. Statistics in Medicine, 15:1217-1225, 1996. **(S00009) (CONCEPT-DRIVEN) (Biometry)**

41. Chen, J.J., Li, L.A., and Jackson, C.D. Analysis of quantal response data from mixture experiments. Environmentrics, 7:503-512, 1996. **(S00009) (CONCEPT-DRIVEN) (Biometry)**

42. Chen, W., Hart, R.W., and Chou, M.W. Effect of dietary restriction on Benzo[a]- pyrene (BaP) metabolic activation and pulmonary BaP-DNA adduct formation in mouse. Drug Chemical Toxicology, 19:21-39, 1996. **(E0260401) (CONCEPT-DRIVEN) (Bio Tox)**

43. Chetty, C.S., Hussain, S.M., Slikker, W., Jr. and Ali, S.F. Effects of phencyclidine on nitric oxide synthase activity in different regions of rat brain. Res. Comm. of Substance Abuse, Accepted: 7/01/96. **(E0683000) (CONCEPT-DRIVEN) (Neuro Tox)**

44. Chou, M.W. and Chen, W. Food restriction reduces AFB_1-DNA adduct formation AFB_1-glutathione conjugation, and DNA damage in AFB_1-treated male F344 rats and B6C3F1 mice. Journal of Nutrition, In Press, 1996. **(E0260401) (CONCEPT-DRIVEN) (Bio Tox)**

45. Chou, M.W., Chen, W., Nichols, J.A., Mikhailova, M.V., and Hart, R.W. Effect of dietary restriction on DNA-adduct formation of benzo[a]pyrene and 2-acetyl- aminofluorene in mouse liver. Polyclic Aromatic Compounds, In Press, 1996. **(E0260401) (CONCEPT-DRIVEN) (Bio Tox)**

46. Chung, K., Murdock, C.A., Zhou, Y., Stevens, S.E., Li, Y.S., Wei, C.I., Fernando, S. Y., and Chou, M. W. Effects of the nitro-group on the mutagenicity and toxicity of some benzamines. Environmental Molecular Mutagenesis, 27:67-74, 1996. **(AGENT-DRIVEN) (Bio Tox)**

47. Churchwell, M.I., Holder, C.L., Preece, S.W., Bajic, S., and Doerge, D.R. Determination of incurred clenbuterol residues in bovine liver using LC-APCI/MS and MS/MS. Proceedings of the 44th ASMS Conference on Mass Spectrometry & Allied Topics, pg. 973, 1996. **(E0694501) (METHOD-DRIVEN) (Chemistry)**

48. Clausing, P.P., Ali, S.F., Newport, G.D., Rybak, S., and Paule, M.G. Central and peripheral neurochemical alterations and immune effects of prenatal ethanol exposure in rats. Int. J. Devel. Neurosci, 14:461-469, 1996. **(E0679600) (CONCEPT-DRIVEN) (Neuro Tox)**

49. Clausing, P.P., Bloom, D.M., Newport, G.D., Holson, R.R., Slikker, W., Jr., and Bowyer, J.F. Individual differences in dopamine release but not rotational behavior correlate with extracellular amphetamine levels in caudate/putamen in unlesioned rats. Psychopharmacology, Accepted: 4/30/96. **(E0690301) (CONCEPT-DRIVEN) (Neuro Tox)**

50. Clausing, P.P., Mothes, H.K., Opitz, B., and Kormann, S. Differential effects of communal rearing and preweaning handling on open field behavior and hot plate latencies in mice. Behavioral Brain Research, Accepted: 4/19/96. **(METHOD-DRIVEN) (Neuro Tox)**

51. Cohn, J., MacPhail, R.C., and Paule, M.G. Repeated acquisition and the assessment of neuroactive compounds. Cognitivie Brain Research, 3:183-191, 1996. **(PREDICTION OF TOXICITY) (Neuro Tox)**

52. Coles B.F. Polypeptides. Virology Methods Manual, 12:251-276, 1996. **(AGENT-DRIVEN) (Bio Tox)**

53. Conte, E.D. and Miller, D.W. A solid phase microextraction-electrodeposition device for the determination of putrescine and cadaverine by high resolution GC. Journal of High Resolution Chromatography, 19:293-297, 1996. **(E0687401) (METHOD-DRIVEN) (Chemistry)**

54. Conte, E.D., Conway, S., Miller, D.W., and Perschbacher, P.W. Determination of methylisoborenol in drinking water and channel catfish pond water by solid phase extraction followed by gas chromatography-mass spectrometry. Water Research, 1996. **(E0687401) (METHOD-DRIVEN) (Chemistry)**

55. Conte, E.D., Perschbacher, P.W., Shen, C.Y., and Miller, D.W. Determination of geosmin and methylisoborneol in catfish tissue (*Ictalurus punctatus*) by microwave assisted distillation-solid phase absorbent trapping. Journal of Agricultural and Food Chemistry, 44:829-835, 1996. **(METHOD-DRIVEN) (Chemistry)**

56. Cooney, C.A., Wise, C.K., and Poirier, L.A. An improved sample preparation method for the quantitative HPLC determination of 5-methyldeoxycytidine in animal tissue DNA. Journal of Liquid Chromatography, Accepted: 10/11/96. **(E0960001) (AGENT-DRIVEN) (Mol Epi)**

57. Culp, S.J. and Beland, F.A. Malachite green: a toxicological review. J. Am. College Tox., 15:219-238, 1996. **(P00345) (METHOD-DRIVEN) (Bio Tox)**

58. Culp, S.J., Gaylor, D.W., Sheldon, W.G., Beland, F.A., and Goldstein, L. DNA adduct measurements in relation to small intestinal and forestomach tumor incidence during the chronic feeding of coal tar or benzo[a]pyrene to mice. Polycyclic Aromatic Compounds, In Press, 1996. **(E0657300) (CONCEPT-DRIVEN) (Bio Tox)**.

59. Dalu, A.N., Cronin, G.M., Lyn-Cook, B.A., and Mehendale, H. Age-related differences in TGF-α and protooncogenes expression in rat liver after a low dose of carbon tetrachloride. J. Biochemical Toxicology, 10(5):259-264, 1996. **(AGENT-DRIVEN)(Mol Epi)**

60. Delclos, K.B., Blaydes, B.J., Heflich, R.H., and Smith, B.A. Assessment of DNA adducts and the frequency of 6-thioguanine resistant T-lymphocytes in F344 rats fed 2,4-toluenediamine or implanted with a toluenedisocyanate-containing polyester polyurethane foam. Mutation Research, 367:209-218, 1996. **(E0677500) (AGENT-DRIVEN) (Bio Tox)**

61. Desai, V.G., Feuers, R.J., and Ali, S.F. MPP+-induced neurotoxicity in mouse is age-dependent: Evidenced by the selective inhibition of complexes of electron transport. Brain Research, 715:1-8, 1996. **(E0683000) (CONCEPT-DRIVEN) (Gen Tox)**

62. Desai, V.G., Weindruch, R., Hart, R.W., and Feuers, R. J. Influences of age and dietary restriction on gastrocenmius electron transport system activities in mice. Archives of Biochemistry and Biophysics, 333(1):145-151, 1996. **(E0683400) (CONCEPT-DRIVEN) (Gen Tox)**

63. Divi, R.L. and Doerge, D.R. Inhibition of thyroid peroxidase by dietary flavonoids. Journal of Chemical Research and Toxicology, 9:16-23, 1996. **(E0692001) (CONCEPT-DRIVEN) (Chemistry)**

64. Doerge, D.R. and Divi, R.L. Porphyrin pi-cation protein radicals in peroxidase catalysis and inhibition by anti-thyroid chemicals. Xenobiotica, 25(7):761-767, 1995. **(CONCEPT-DRIVEN) (Chemistry)**

65. Doerge, D.R. and Bajic, S. Multiresidue determination of quinolone antibiotics using LC-APCI/MS and MS/MS. Rapid Communications in Mass Spectrometry, Vol. 9, 1995. **(METHOD-DRIVEN) (Chemistry)**

66. Doerge, D.R., Bajic, S., Blankenship, L., Preece, S.W., and Churchwell, M.I. Determination of β-agonist residues in human plasma using LC-APCI and MS/MS. Proceedings of the American Society for Mass Spectrometry, pg. 330, 1995. **(AGENT-DRIVEN) (Chemistry)**

67. Doerge, D.R., Bajic, S., Blankenship, L., Preece, S.W., and Churchwell, M.I. Determination of β-agonist residues in human plasma using LC-APCI/MS. Journal of Mass Spectrometry, 30:911, 1995. **(AGENT-DRIVEN) (Chemistry)**

68. Doerge, D.R., Churchwell, M.I., Holder, C.L., Rowe, L., and Bajic, S. Determination and confirmation of β-agonists in bovine retina using LC-APCI/MS. Analytical Chemistry, 68:1918-1923, 1996. **(E0694501) (METHOD-DRIVEN) (Chemistry)**

69. Doerge, D.R., Churchwell, M.I., Rushing, L.G., and Bajic, S. Multiresidue confirmation of gentian violet and leucogentian violet in catfish muscle using LC-APCI/MS. Proceedings of 44th ASMS Conference on Mass Spectrometry, pg. 872, 1996. **(E0211901) (METHOD-DRIVEN) (Chemistry)**

70. Doerge, D.R., Churchwell, M.I., Rushing, L.G., and Bajic, S. Confirmation of gentian violet and its metabolite leucogentian violet in catfish muscle using LC-APCI/MS. Rapid Communications in Mass Spectrometry. Accepted: 8/22/96. **(E0211901) (METHOD-DRIVEN) (Chemistry)**

71. Doerge, D.R., Holder, C.L., and Preece, S.W. Quantification of heterocyclic amine carcinogens in cooked meats using LC-APCI/MS/MS. Proceedings of the 44th ASMS Conference on Mass Spectrometry & Allied Topics, pg. 971, 1996. **(METHOD-DRIVEN) (Chemistry)**

72. Duffy, P.H., Feuers, R.J., Pipkin, J.L., Turturro, A., and Hart, R.W. Age and temperature related changes in behavioral and physiological changes in the *peromyscus leucopus* mouse. Mechanisms of Ageing and Development. In Press, 1996. **(CONCEPT-DRIVEN) (Cal Res)**

73. Duffy, P.H., Leakey, J.E., Pipkin, J.L., Turturro, A., and Hart, R.W. The neurophysiological and behavioral response to a low calorie diet: Implications for environmental and health effects. Journal of Environmental Medicine. 10/09/96. **(E0678800) (AGENT-DRIVEN) (Cal Res)**

74. Ferguson, S. A., Paule, M. G. Effects of chlorpromazine and diazepam on time estimation behavior and motivation in rats. Pharmacology, Biochemistry and Behavior, 53(1):115-122, 1996. **(E0673000) (PREDICTION OF TOXICITY) (R&D Tox)**

75. Ferguson, S.A. A review of the neuroanatomical and functional alterations resulting from early postnatal cerebellar insults in rodents. Pharmacology Biochemistry & Behavior, Accepted: 5/20/96. **(CONCEPT-DRIVEN) (R&D Tox)**

76. Ferguson, S.A. and Holson, R.R. Methylazoxymethanol-induced micrencephaly in the Brown Norway strain: Behavior and brain weight. Int J Developmental Neuroscience, Accepted: 2/26/96. **(E0690401) (PREDICTION OF TOXICITY) (R&D Tox)**

77. Ferguson, S.A., Felipa, H.N., and Bowman, R.E. Effects of acute treatment with dopaminergic drugs on open field behavior of adult monkeys treated with lead during the first year postpartum. Neurotoxicology and Teratology, 18:181-188, 1996. **(AGENT-DRIVEN) (R&D Tox)**

78. Ferguson, S.A., Paule, M.G., and Holson, R.R. Functional effects of methylazoxymethanol-induced hypoplasia cerebellar lesions in rats. Neurotoxicology and Teratology, 18(5):529-537, 1996. **(CONCEPT-DRIVEN) (R&D Tox)**

79. Ferguson, S.A., St. Omer, V.E., Kwon, O.S., Holson, R.R., Houston, R.J., Rottinghaus, G.E., and Slikker, W., Jr. Prenatal fumonisin (FB_1) treatment in rats results in minimal maternal or offspring toxicity. Neurotoxicology, Accepted: 5/29/96. **(E0211511) (AGENT-DRIVEN) (R&D Tox)**

80. Fishman, R.B., Branham, W.S., Streck, R.D., and Sheehan, D.M. Ontogeny of estrogen receptor mRNA expression in the postnatal rat uterus. Biology of Reproduction, Accepted: 7/25/96. **(E0688601) (Knowledge-Base) (R&D Tox)**

81. Frederick, D.L. and Paule, M.G. Effects of MDMA on complex brain functions in laboratory animals. Neuroscience and Behavioral Reviews, In Press, 1996. **(E0694300) (AGENT-DRIVEN) (Neuro Tox)**

82. Frederick, D.L., Gillam, M.P., Lensing, S.Y., and Paule, M.G. Acute effects of LSD on rhesus monkey operant test battery performance. Pharmacology, Biochemistry and Behavior, Accepted: 2/26/96. **(E0644300) (KNOWLEDGE BASE) (Neuro Tox)**

83. Freni, S.C., Ebrehardt, M., Turturro, A., and Hine, R. Anthropometric measures and metabolic rate in association with breast cancer. Cancer Causes and Control, 7:358-365, 1996. **(E0687101) (AGENT-DRIVEN) (Biometry)**

84. Freyaldenhoven, T.E., Cadet, J., and Ali, S.F. The neurotoxic effects of 1-methyl-4-phenyl-1,2,3,6- tetrahydropyridine in CD-1 mice are gender-dependent. Brain Research, Accepted: 7/20/96. **(E0688701) (CONCEPT-DRIVEN) (Neuro Tox)**

85. Fu, P.P, Von Tungeln L.S, Zhan D.J., and Bucci T. Potent tumorigenicity of 7-chlorobenz[a]anthracene and 7-bromobenz[a]anthracene in the neonatal $B_6C_3F_1$ male mouse. Cancer Letters, 101:37-42, 1996. **(E0657300) (PREDICTIVE TOXICITY) (Bio Tox)**

86. Fu, P.P., Qui, F.Y., Jung, H., Von Tungeln, L.S., Zhan, D., Lee, M., Wu, Y., and Heflich, R.H. Metabolism of isomeric nitrobenzo[a]pyrenes leading to DNA adducts and mutagenesis. Mutation Research, In Press, 1996. **(E0657300) (PREDICTIVE TOXICITY) (Bio Tox)**

87. Fu, P.P., Zhan, D., Von Tungeln, L.S., Yi, P., Qui, F.-Y., Herreno-Saenz, D., and Lewtas, J. Comparative formation of DNA adducts of nitro-polycyclic aromatic hydrocarbons in mouse and rat liver microsomes and cytosols. Polycyclic Aromatic Compounds, In Press, 1996. **(E0657300) (PREDICTIVE TOXICITY) (Bio Tox)**

88. Gaylor, D.W. and Zheng, Q. Risk assessment of nongenotoxic carcinogens based upon cell proliferation/death rates in rodents. Risk Analysis, 16(2):221-225, 1996. **(S00174) (METHOD-DRIVEN) (Biometry)**

89. Gaylor, D.W. and Chen, J.J. A simple upper limit for the sum of the risks of the components in a mixture. Risk Analysis, 16(3):395-398, 1996. **(E0691001) (PREDICTIVE TOXICITY) (Biometry)**

90. Gaylor, D.W. and Chen, J.J. Precision of benchmark dose estimates for continuous (Nonquantal) biological endpoints. Regulatory Toxicology and Pharmacology, 24:19-23, 1996. **(S00116) (CONCEPT-DRIVEN) (Biometry)**

91. Gaylor, D.W. Quantalization of continuous data for benchmark dose estimation. Regulatory Toxicology and Pharmacology. Accepted: 8/20/96. **(METHOD-DRIVEN) (Biometry)**

92. Gaylor, D.W. Risk Estimation - An Overview: Disease risk estimation based upon animal bioassays. Drug Metabolism Reviews, 28:9-27, 1996. **(S00116) (CONCEPT-DRIVEN) (Biometry)**

93. Gaylor, D.W. Some current procedures and issues in cancer risk assessment. Human and Ecological Risk Assessment, Vol. 2, 1996. **(S00116) (CONCEPT-DRIVEN) (Biometry)**

94. Gehring, T.A., Rushing, L.G., Churchwell, M.I., Doerge, D.R., and Thompson, H.C. HPLC determination of sulfadiazine residues in coho salmon (*oncorhyncus kisutch*) with confirmation by LC-APCI/MS. Journal of Agricultural and Food Chemistry, 44(9):3164-3169, 1996. **(METHOD-DRIVEN) (Chemistry)**

95. George, E. and Kodell, R.L. Tests of independence, treatment heterogeneity and dose-related trend with exchangeable binary data. Journal of the American Statistical Association. Accepted: 3/07/96. **(METHOD-DRIVEN) (Biometry)**

96. Greenman, D.L., Morrissey, R.L., Blakemore, W., and Crowell, J. Subchronic toxicity of Triethylenetetramine dihydrochloride in B6C3F1 mice and F344 rats. Fundamental and Applied Toxicology, 29:185-193, 1996. **(E0043200) (AGENT-DRIVEN) (Research)***

97. Gu, Y., Srivastava, R.K., Clarke, D.L., Linzer, D.I., and Gibori, G. The decidual prolactin receptor and its regulation by decidua-derived factors. Endocrinology, Accepted: 7/18/96. **(CONCEPT-DRIVEN) (R&D Tox)**

98. Halsted, C., Villanueva, J., Chandler, C., Stabler, S., Muskhelishvili, L., James-Gaylor, S.J., and Poirier, L.A. Ethanol feeding of micropigs alters methioninie metabolism and increases hepatocellular apoptosis and proliferation. Hepatology, 23:497-505, 1996. **(AGENT-DRIVEN) (Bio Tox)**

99. Hansen, D.K., Dial, S.L., Terry, K.K., and Grafton, T.F. In vitro embryotoxicity of carbamazepine and carbamazepine-10,11-expoxide. Teratology, Accepted: 4/04/96, In Press. **(E0688101) (AGENT-DRIVEN) (R&D Tox)**

100. Hansen, E., Churchwell, M.I., Zhang, D., Cerniglia, C.E., and Doerge, D.R. HPLC-APCI analysis of the antihistamines cyproheptadine and azatadine and their microbial metabolites. 44th Annual ASMS Conference on Mass Spectrometry and Allied Topics, pg. 254, 1996. **(E0698001) (METHOD-DRIVEN) (Chemistry)**

101. Hansen, E., Luo, W., Churchwell, M.I., Thompson, H.C., and Doerge, D.R. Investigation of the HPLC electrochemical response of erythromycin by HPLC-ECD-APCI analysis. Proceedings of 44th ASMS Conference on Mass Spectrometry and Allied Topics, pg. 178, 1996. **(E0698001)(AGENT-DRIVEN)(Chemistry)**

102. Harris, E.M., Syvertson, C.N., and Thompson, H.C. Evaluation of two methods for the determination of residues of fenbutatin oxide in apples, oranges, and cucumbers. Laboratory Information Bulletin, 12#1:LIB#4009, 1996. **(E0677300) (METHOD-DRIVEN) (Chemistry)**

103. Hart, R.W. and Frame, L.T. Toxicological defense mechanisms and how they may affect the nature of dose-response relationships. BELLE Newsletter, 5:1, 1996. **(CONCEPT-DRIVEN) (Cal Res)**

104. Hart, R.W. and Turturro, A. Evolution and dietary restriction. Experimental Gerontology, In Press, 1996. **(CONCEPT-DRIVEN) (Cal Res)**

105. Hart, R.W. and Turturro, A. Dietary restrictions and cancer. Monograph to the Lovelace Institute's 1995 Annual Symposium "Mechanisms and Prevention of Environmentally Caused Cancers. Accepted: 9/04/96. **(E0050800) (CONCEPT-DRIVEN) (Cal Res)**

106. Hart, R.W. and Turturro, A. Is there a new cancer risk assessment paradigm needed? BELLE Newsletter, Vol. 5, No. 2/3, pp. 10-11, 1996. **(CONCEPT-DRIVEN) (Cal Res)**

107. Hart, R.W., Hass, B.S., Lewis, S.M., and Turturro, A. The use of nutrition to implement refinement in animal bioassays. Lab Animal, 25:29-34, 1996. **(CONCEPT-DRIVEN) (Cal Res)**

108. Hart, R.W., Leakey, J.E., Duffy, P.H., Feuers, R.J., and Turturro, A. The effects of dietary restriction on drug testing and toxicity. Exp Toxic Pathol, 48:121-127, 1996 **(E0050800) (CONCEPT-DRIVEN) (Cal Res)**

109. Hashemi, R.R., Pearce, B.A., Arani, R.B., Hinson, W.G., and Paule, M.G. A hybrid system for knowledge elicitation. Proceedings of World Automation Conference '96, Accepted: 3/15/96. **(E0663800) (METHOD-DRIVEN) (Neuro Tox)**

110. Hass, B.S. and Littlefield, N.A. Cytotoxicology and the three R's. In: Introduction to In Vitro Cytotoxicology: Mechanisms and Methods. pgs.. 51-53, 1996. F.A. Barile, ed., CRC Press, Inc. **(METHOD-DRIVEN) (R&D Tox)**

111. Hass, B.S., Lewis, S.M., Lipschitz, D., and Hart, R.W. Dietary restriction in humans: Report on the Little Rock Conference on the value, feasibility, and parameters of a proposed study. Mechanisms of Ageing and Development, Accepted: 7/06/96. **(CONCEPT-DRIVEN) (R&D Tox)**

112. Hass, B.S., McDaniel, L.P., and Littlefield, N.A. Actions and interactions of nickel and magnesium on the transformation response of transformed cells in culture. Annals of Clinical and Laboratory Science, 26(1):18-30, 1996. **(CONCEPT-DRIVEN) (R&D Tox)**

113. Heflich, R.H., Mittelstaedt, R.A., Manjanatha, M., Lyn-Cook, L.E., and Aidoo, A. DNA sequence analysis of hprt mutations in lymphocytes from Sprague-Dawley rats treated with 7,12-dimethylbenz[a]anthracene. Environmental and Molecular Mutagenesis, 28:5-12, 1996. **(E0685000) (PREDICTION OF TOXICITY) (Gen Tox)**

114. Hendry, B., Zheng, X., Leavitt, W.W., Branham, W.S., and Sheehan, D.M. Synthetic estrogen-mediated alterations in uterine cell fate. Serono Symposium: Cell Death in Reproductive Physiology, Accepted: 7/31/96. **(CONCEPT-DRIVEN) (R&D Tox)**

115. Holcomb, M. and Thompson, H.C. Analysis of fumonisin B_1 in rodent feed by capillary electrophoresis with fluorescence detection of the FMOC derivative. J. Capillary Electrophoresis, 3(4), 1996. **(P00321) (AGENT-DRIVEN) (Chemistry)**

116. Holcomb, M., Hopper, M.L., Cooper, W.M., and Thompson, H.C. Extraction of aflatoxins (B_1, B_2, G_1, & G_2) from corn by SFE and analysis by HPLC. Proceedings of the 3rd International Symposium on Supercritical Fluids, Accepted: 9/30/96 **(METHOD-DRIVEN) (Chemistry)**

117. Holcomb, M., Hopper, M.L., Cooper, W.M., and Thompson, H.C. SFE extraction of aflatoxins (B_1, B_2, G_1, and G_2) from corn and analysis by HPLC. The Journal of Supercritical Fluids, 9:118-121, 1996. **(METHOD-DRIVEN) (Chemistry)**

118. Holder, C.L., Cooper, W.M., Churchwell, M.I., Doerge, D.R., and Thompson, H.C. Multi-residue determination and confirmation of ten heterocyclic amines in cooked meats. Journal of Muscle Foods, 7(3):281-291, 1996. **(METHOD-DRIVEN) (Chemistry)**

119. Holland, R.D., Persons, C.C., Heinze, T.M., Shaikh, A.U., and Lay, J.O. Protein ladder sequencing peptides containing lysine after enzymatic digestion using MALDI/TOF MS. Proceedings of ASMS, pg. 357, 1996. **(E0678500) (METHOD-DRIVEN) (Chemistry)**

120. Holland, R.D., Wilkes, J.G., Rafii, F., Sutherland, J.B., Persons, C.C., Voorhees, K., and Lay, J.O. Rapid identification of intact whole bacteria based on spectral patterns using MALDI/TOF MS. Proceedings of the 44th ASMS Conference on Mass Spectrometry, pg. 994, 1996. **(E0678500) (METHOD-DRIVEN) (Chemistry)**

121. Holson, R.R., Bowyer, J.F., Clausing,P.P., and Gough, B.J. Methamphetamine-stimulated striatal dopamine release declines rapidly over time following microdialysis probe insertion. Brain Research, Accepted: 7/02/96 **(E0690301) (CONCEPT-DRIVEN) (R&D Tox)**

122. Hsiao, W.L., Wolff, G.L., Barsh, G.L., North, B. M., and Fan, H. Differential spontaneous transformation *in vitro* of newly established mouse fibroblast lines carrying or lacking the viable yellow mutation (Avy) of the mouse agouti locus. Cancer Research, 15(1):70-80, 1996. **(E0668200) (CONCEPT-DRIVEN) (Bio Tox)**

123. Huber, W., McDaniel,.P., Kaderlik, K., Teitel, C.H., Lang, N.P. and Kadlubar, F.F. Chemoprotection against the formation of colon DNA adducts from the food-borne carcinogen, 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine (PhIP) in the rat. Mutation Research, Accepted: 5/19/96. **(E0689401) (PREDICTIVE TOXICITY) (Mol Epi)**

124. Hussain, S.M., Slikker, W., Jr., and Ali, S.F. Role of medallothionein and other antioxidants in scavenging superoxide radicals and their possible role in neuroprotection. Neurochemistry International, 29, No. 2:145-152, 1996. **(E0671900) (AGENT-DRIVEN) (Neuro Tox)**

125. Jackson, C.D., Cronin, G.M., and Hsu, H.H. Subchronic studies of triprolidine in B6C3F1 mice. Drug and Chemical Toxicology, Accepted: 9/30/96. **(E0043100) (AGENT-DRIVEN) (R&D Tox)**

126. Jackson, C.D., Weis, C.C., Poirier, L.A., and Bechtel, D.H. Interactions of varying levels of dietary fat, carbohydrate, and fiber on food consumption and utilization, weight gain and fecal fat contents in female. Nutrition Research, Accepted: 5/21/96. **(E0674600) (CONCEPT-DRIVEN) (R&D Tox)**

127. Jackson, C.D., Weis, C.C., Miller, B.J., and James-Gaylor, S.J. Dietary nucleotides: Effects on cell proliferation following partial hepatectomy in rats fed NIH-31, AIN-76A, or folate/methyl deficient diets. Journal of Nutrition, Accepted: 8/26/96. **(E0678000) (CONCEPT-DRIVEN) (R&D Tox)**

128. James-Gaylor, S.J., Miller, B.J., Basnakian, A.G., Pogribna, M.V., Pogribny, I.P., and Muskhelishvili, L. Apoptosis and regenerative proliferation under conditions of deoxynucleotide pool imbalance in liver of folate/methyl deficient rats. Carcinogenesis, In Press, 1996. **(CONCEPT-DRIVEN) (R&D Tox)**

129. Kadlubar, F.F., Young, J.F., Lang, N.P., Caporaso, N.E., Sinha, R., and Landi, M. T. CYP1A2 phenotyping using caffeine. Cancer Epidemiol., Biomarkers & Prev., 5:757-758, 1996. **(E0694700) (Predictive Toxicity) (Mol Epi)**

130. Khan, A.A. and Cerniglia, C.E. Rapid and sensitive method for the detection of *Aeromonas caviae* and *Aeromonas trota* by PCR. Letters in Applied Microbiology. Accepted: 7/29/96. **(E0690001) (METHOD-DRIVEN) (Micro)**

131. Khan, A.A., Nawaz, M.S., and Cerniglia, C.E. Purification of 2,3-dihydroxybiphenyl 1,2 dioxygenase from *Pseudomonas putida* OU83 and characterization of the gene (bphC). Applied and Environmental Microbiology, 62:1825-1830, 1996. **(METHOD-DRIVEN) (Micro)**

132. Khan, A.A., Wang, R., Wei, C.I., Franklin, W., and Cerniglia, C.E. Reclassification of a Polycyclic Aromatic Hydrocarbon-metabolizing bacterium, *Beijerinckia sp.* strain B1, as *Sphingomonas yanoikuyae* by fatty acid analysis, protein pattern analysis, DNA-DNA hybridization, and 16S ribosomal DNA sequencing. International J. of Systematic Bacteriology, 46:466-469, 1996. **(E0690001) (METHOD-DRIVEN) (Micro)**

133. Kim, C., Binienda, Z.K., and Sandberg, J.A. Construction of a physiologically based pharmacokinetic model for 2,4-dichlorophenoxyacetic acid (2,4-D) dosimetry in the developing rabbit brain. Toxicology and Applied Pharmacology, 136:250-259, 1996. **(E0681500) (PREDICTION OF TOXICITY) (Neuro Tox)**

134. Kim, Y. I., Pogribny, I., Basnakian, A. G., Miller, J. W., Selhub, J. J., James-Gaylor, S. J., and Mason, J. B. Exon-specific DNA hypomethylation of p53 gene of rat colon induced by dimethylhydrazine: modulation by dietary folate. American Journal of Pathology, In Press, 1996. **(CONCEPT-DRIVEN) (Bio Tox)**

135. Kim, Y.I., Pogribny, I.P., Basnakian, A.G., Miller, J.W., Selhub, J.J., James-Gaylor, S. J., and Mason, J.B. Folate deficiency in the rat induces DNA strand breaks and hypomethylation within the p53 tumor suppressor gene. Journal of Clinical Nutrition, In Press, 1996. **(CONCEPT-DRIVEN) (Bio Tox)**

136. Kodell, R.L. and Ahn, H. Age-adjusted trend test for the tumor incidence rate. Biometrics. Accepted: 8/31/96. **(E0687001) (METHOD-DRIVEN) (Biometry)**

137. Kodell, R.L. and Ahn, H. Nonparametric trend test for the cumulative tumor incidence rate. Communication in Statistics - Theory and Methods, 25(8):1677-1692, 1996. **(E0687001) (METHOD-DRIVEN) (Biometry)**

138. Kodell, R.L. and Gaylor, D.W. Uncertainty of estimates in extrapolation of cancer risks from high to low dose. Journal American College of Toxicology. Accepted: 6/10/96. **(S00116) (CONCEPT-DRIVEN) (Biometry)**

139. Kodell, R.L., Basu, A., and Gaylor, D.W. On interspecies correlations of carcinogenic potencies. Journal of Toxicology and Environmental Health, 48:101-107, 1996. **(S00116) (CONCEPT-DRIVEN) (Biometry)**

140. Kodell, R.L. Bioassay designs for validating biologically based mathematical models of carcinogenesis for risk assessment. Drug Metabolism Reviews, 28(1&2):219-223, 1996. **(S00116) (CONCEPT-DRIVEN) (Biometry)**

141. Kwon, O., Sandberg, J.A., and Slikker, W., Jr. Effects of fumonisin B1 treatment on blood-brain barrier transfer in developing rats. Neurotoxicology and Teratology, Accepted: 10/23/96. **(E0688401) (AGENT-DRIVEN) (Neuro Tox)**

142. Kwon, O., Schmued, L.C., and Slikker, W., Jr. Fumonisin B1 treatment in developing rats directly alters brain sphinganine levels and mimics the hypomyelination toxicity induced by limited nutrition. NeuroToxicology, Accepted: 10/30/96. **(E0688401) (AGENT-DRIVEN) (Neuro Tox)**

143. Laborde, J.B., Bates, H.K., Dacre, J.C., and Young, J.F. Developmental toxicity of sarin in rats and rabbits. J Toxicology Environmental Health, 47:249-265, 1996. **(E0616401) (AGENT-DRIVEN) (R&D Tox)**

144. Landi, M.T., Zocchetti, C., Bernucci, I., Kadlubar, F.F., Tannenbaum, S., Skipper, P., Bartsch, H., Malaveille, C., Shields, P.G., and Caporaso, N.E. Cytochrome P4501A2: Enzyme induction and genetic control in determining 4-aminobi-phenyl-hemoglobin adduct levels. Cancer Epidemiology, Biomarkers and Prevention, Accepted: 6/4/96. **(E0674700) (Predictive Toxicity) (Mol Epi)**

145. Lay, J.O. Book Review: Mass Spectrometry in Biomolecular Sciences. Journal of the American Society for Mass Spectrometry, pg. 45, 1996. **(E0678500) (METHOD-DRIVEN) (Chemistry)**

146. Lay, J.O., Holland, R.D., Wilkes, J.G., Rafii, F., Sutherland, J.B., Persons, C.C., and Voorhees, K. Rapid identification of intact whole bacteria based on spectral patterns using MALDI/TOF MS. Rapid Communications in Mass Spectrometry, 10:1227-1232, 1996. **(E0678500) (METHOD-DRIVEN) (Chemistry)**

147. Lay, J.O., Musser, S.M., Chiarelli, M.P., and Wilkes, J.G. FAB/MS/MS and HPLC/ESI/MS/MS of fumonisins B_2 and B_3. Journal of Chromatography, 1:278-285, 1996. **(E0678500) (METHOD-DRIVEN) (Chemistry)**

148. Lin, S.D., Jih, Y.-P., and Fu, P.P. Mass spectral analysis of nitro-polycyclic aromatic hydrocarbons with torsion angle obtained from semiempirical calculations. J. Org. Chem., 61:5271-5273, 1996. **(E0657300) (PREDICTIVE TOXICITY) (Bio Tox)**

149. Littlefield, N.A., Gaylor, D.W., and Hass, B.S. Magnesium protects against DNA damage from specific heavy metals. Metal Ions in Biology and Medicine. Proceedings of the 4th International Symposium. Current Research in Magnesium, Chapter 23, pgs. 95-99, 1996. **(CONCEPT-DRIVEN) (Bio Tox)**

150. Littlefield, N.A., Sheldon, W.G., Lyn-Cook, B.A., and Hass, B.S. The effect of dietary magnesium on the transformation process in mice. Proceedings of the 9th International Symposium on Trace Metals in Animals and Man TEMA-9, In Press, 1996. **(E0684800) (AGENT-DRIVEN) (Bio Tox)**

151. Liu, F., Ang, C.Y., Huang, Y.W., and Toledo, R.T. Fat/water composition affects residual catalitic activity in chicken breast heated to specific end-point temperature. Journal of Food Science, Accepted: 6/12/96. **(E0693601) (METHOD-DRIVEN) (Chemistry)**

152. Liu, F., Ang, C.Y., Toledo, R.T., and Huang, Y.W. Severity of heat treatment affects hydroperoxidase activity in cooked chicken breast meat. Journal of Food Science, 6(1):213-216, 1996. **(E0693601) (METHOD-DRIVEN) (Chemistry)**

153. Long, S., Chowdhury, S.M., Ali, S.F., Matthews, J.C., and Wilson, M.C. Differential effects of dominance status on sensitivity to cocaine in male rats. Pharmacology Biochemistry Behavior, Accepted: 9/20/96. **(CONCEPT-DRIVEN) (Neuro Tox)**

154. Luo, W., Hansen, E., Ang, C.Y., and Thompson, H.C. Determination of lincomycin residue in salmon tissues by ion pair reverse phase HPLC with electrochemical detection. Journal of AOAC International, 79(4):839-843, 1996. **(E0693601) (METHOD-DRIVEN) (Chemistry)**

155. Luo, W., Yin, B., Ang, C.Y., Rushing, L.G., and Thompson, H.C. Determination of lincoymcin residue in salmon tissues by gas chromatography with nitrogen/phosphorous specific detector. Journal of Chromatography, Accepted: 5/21/96. **(E0693601) (METHOD-DRIVEN) (Chemistry)**

156. Lutz, W. and Gaylor, D.W. Significance of DNA adducts at low dose: shortening the time to spontaneous tumor occurrence. Regulatory Toxicol. Pharmacology, 23:29-34, 1996. **(CONCEPT-DRIVEN) (Biometry)**

157. Lyn-Cook, B.A., Montague, D., Poirier, L.A. and Littlefield, N.A. Metal interactions in chemical carcinogenesis. Toxicology of Metals, Chapter 17:289-298, 1996. Louis W. Chang, ed., Lewis Publishers of CRC Press., Boca Raton, Florida. **(E0696700) (AGENT-DRIVEN) (Mol Epi)**

158. Manjanatha, M., Li, E., Fu, P.P., and Heflich, R.H. Comparison of *ras* mutations produced in liver tumors from B6C3F1 and CD-1 mice treated with benzo(A)pyrene, 6-nitrochrysene and 4-aminobiphenyl. Journal of Toxicology and Environmental Health, 47:195-208, 1996. **(E0679200) (METHOD-DRIVEN) (Gen Tox)**

159. Manjanatha, M., Lyn-Cook, L.E., Culp, S.J., Beland, F.A., Heflich, R.H., and Aidoo, A. Lymphocyte mutant frequency in relation to DNA adduct formation in rats treated with tumorigenic doses of the mammary carcinogen 7,12-dimethylbenz[a]anthracene. Mutation Research, 357:89-96, 1996. **(E0685000) (PREDICTION OF TOXICITY) (Gen Tox)**

160. Marques, M.M., Mourato, L.L.G., Santos, M.A., and Beland, F.A. Synthesis, characterization, and conformation analysis of DNA adducts from methylated anilines present in tobacco smoke. Chemical Research in Toxicology, 9:99-108, 1996. **(E0673700) (PREDICTIVE TOXICITY) (Bio Tox)**

161. Matthews, A.M., Hinson, J.A., Roberts, D.W., and Pumford N.R. Comparison of covalent binding of acetaminophen and the regioisomer 3'-hydroxyacetanilide to mouse liver protein. Tox. Letters, In Press, 1996. **(AGENT-DRIVEN) (Bio Tox)**

162. Matthews, A.M., Roberts, D.W., Hinson, J.A., and Pumford, N.R. Acetaminophen-induced hepatotoxicity: analysis of total covalent binding versus specific binding to cysteine. Drug Met. Disp., In Press. **(AGENT-DRIVEN) (Bio Tox)**

163. Medlock, K.L., Branham, W.S., and Sheehan, D.M. The effects of toremifene on neonatal rat uterine growth and differentiation. Biology of Reproduction, Accepted: 9/30/96. **(E0688501) (KNOWLEDGE-BASE) (R&D Tox)**

164. Morris, P.D., Binienda, Z.K., Gillam, M.P., Klein, J., McMartin, K., Koren, G., Duhart, H.M., Slikker, W., Jr., and Paule, M.G. The effects of chronic cocaine exposure throughout pregnancy on maternal and infant outcomes in the rhesus monkey. Neurotoxicology and Teratology, Accepted: 9/10/96. **(E0663300) (AGENT-DRIVEN) (Neuro Tox)**

165. Morris, P.D., Binienda, Z.K., Harkey, M.R., Gillam, M.P., Paule, M.G., Zhou, C., and Henderson, G.L. The effect of chronic cocaine exposure during pregnancy on maternal and infant outcomes in the rhesus monkey. Neurotoxicology and Teratology, 18:147-154, 1996. **(E0663300) (AGENT-DRIVEN) (Neuro Tox)**

166. Morris, P.D., Gillam, M.P., Allen, R.R., and Paule, M.G. The effect of chronic cocaine exposure during pregnancy on the acquisition of operant behaviors by rhesus monkey offspring. Neurotoxicology and Teratology, 18(2):155-166, 1996. **(E0663300) (AGENT-DRIVEN) (Neuro Tox)**

167. Morris, S.M., Domon, O.E., McGarrity, L.J., Chen, J.J., Manjanatha, M., Andrews, A.M., and Aidoo, A., Casciano, D. A. A role for apoptosis in the toxicity and mutagenicity of Bleomycin in AHH-1 tk+/- human lumphoblastoid cells. Mutation Research, 357:143-165, 1996. **(E0674100) (CONCEPT-DRIVEN) (Gen Tox)**

168. Morris, S.M., Manjanatha, M., Shelton, S.D., Domon, O.E., McGarrity, L.J., and Casciano, D. A. A mutation in the p53 tumor suppressor gene of AHH-1 tk+/- human lymphoblastoid cells. Mutation Research, 356:129-134, 1996. **(E0674100) (AGENT-DRIVEN) (Gen Tox)**

169. Morris, S.M., McGarrity, L.J., Domon, O.E., Chen, J.J., and Casciano, D.A. Cell-cycle traverse in AHH-1 human lymphoblastoid cells exposed to the chromosomal mutagen, m-amsa. Environmental and Molecular Mutagenesis, 27:10-18, 1996. **(E0674100) (CONCEPT-DRIVEN) (Gen Tox)**

170. Muskhelishvili, L., Turturro, A., Hart, R.W., and James-Gaylor, S.J. PI-class glutathionine-S- transferase-positive hepatocytes undergo apoptosis induced by dietary restriction. American Journal of Pathology, In Press, 1996. **(CONCEPT-DRIVEN) (Bio Tox)**

171. Nawaz, M.S., Khan, A.A., Bhattacharyya, D., Siitonen, P.H., and Cerniglia, C.E. Physical, biochemical and immunological characterization of a thermostable amidase from *Klebsiella pneumoniae* NCTR 1. J. Bacteriology, 178:2397-2401, 1996. **(E0680900) (KNOWLEDGE BASE) (Micro)**

172. Ni, Y., Von Tungeln, L.S., Kadlubar, F.F., and Fu, P.P. Formation of chloral hydrate, trichloroacetic acid and trichloroethanol induced malondialdehyde-modified DNA adduct mediated by mouse, rat, and human liver microsomes. Drug Met. Disp., In Press, 1996. **(E02100) (AGENT-DRIVEN) (Bio Tox)**

173. Ni, Y.-C., Wong, T.-Y., LLoyd, R.V., Henize, T.M., Shelton, S., Casciano, D., Kadlubar, F.F., and Fu, P.P. Mouse liver microsomal metabolism of chloral hydrate, trichloroacetic acid and trichloroethanol leading to induction of lipid peroxidation via a free radical mechanism. Drug Met. Disp., 24:81-90, 1996. **(E02100) (AGENT-DRIVEN) (Bio Tox)**

174. Noah, C., Poteet, S., Lister, M., Roderick, C., Smith, D.B., Colvert, R.M., Holland, M. A., and Cerniglia, C.E. Production and characterization of monoclonal antibodies to detect *Vibrio cholerae* serogroup 01 in a rapid enzyme-linked immunosorbent assay. Journal of Food Protection, Accepted: 3/25/96 **(E0684400) (METHOD-DRIVEN) (Micro)**

175. Opitz, B., Mothes, H.K., and Clausing, P.P. Effects of prenatal ethanol exposure and early experience on radial maze performance and conditioned taste aversion in mice. Neurotoxicology and Teratology, Accepted: 9/30/96. **(CONCEPT-DRIVEN) (Neuro Tox)**

176. Parsons, B.L. and Heflich, R.H. Evaluation of MutS as a tool for direct measurement of point mutations in genome. Mutation Research, Accepted: 7/19/96. **(E0693501) (METHOD-DRIVEN) (Gen Tox)**

177. Patterson, T.A., Eppler, B., and Dawson, R. Attenuation of trimethyltin-evoked glutamate (GLU) efflux from rat cortical and hippocampal slices. Neurotoxicology and Teratology, Accepted: 6/07/96. **(CONCEPT-DRIVEN) (Neuro Tox)**

178. Paule, M.G., Gillam, M.P., Binienda, Z.K., and Morris, P.D. Chronic cocaine exposure throughout gestation in the rhesus monkey: Pregnancy outcomes and offspring behavior. Annals of the New York Academy of Sciences, Accepted: 5/01/96. **(E0663300) (AGENT-DRIVEN) (Neuro Tox)**

179. Pipkin, J.L., Hinson, W.G., Lyn-Cook, L.E., Duffy, P.H., Feuers, R.J., Leakey, J.E., Aly, K.B., Hart, R.W., and Casciano, D.A. The effect of aging and dietary restriction on the retinoylation of nuclear proteins in rats. Aging, In Press, 1996. **(E0671700) (CONCEPT-DRIVEN) (Gen Tox)**

180. Poirier, L.A. Current Issues: FDA Workshop on Indirect Mechanisms of Carcinogenesis. Toxicologic Pathology, 24(5):659-661, 1996. **(P00368) (PREDICTION OF TOXICITY) (Mol Epi)**

181. Poirier, L.A. Selected concepts in metal carcinogenesis: Introduction to Book Chapter, Toxicology of Metals, pgs. 287-288, 1996. L.W. Chang, ed., Lewis Publishers. **(E0696700) (AGENT-DRIVEN) (Mol Epi)**

182. Poirier, M., Beland, F.A. Aromatic amine DNA Adduct formation in chronically-exposed rodents: considerations for human risk assessment. Mutation Research, In Press, 1996. **(PREDICTIVE TOXICITY) (Bio Tox)**

183. Pothuluri, J.V., Evans, F.E., Heinze, T.M., and Cerniglia, C.E. Formation of sulfate and glucoside conjugates of benzo[e]pyrene by *Cunninghamella elegans*. Appl Microbiol Biotechnol, 45:677-683, 1996. **(E0672300) (KNOWLEDGE BASE) (Micro)**

184. Pothuluri, J.V., Evans, F.E., Doerge, D.R., Churchwell, M. I., and Cerniglia, C.E. Metabolism of metolachlor by the fungus *Cunninghamella elegans*. Archives of Environmental Contamination and Toxicology. Accepted: 5/11/96 **(E0690101) (METHOD-DRIVEN) (Micro)**

185. Pothuluri, J.V., Evans, F.E., Heinze, T.M., Fu, P.P., and Cerniglia, C.E. Fungal metabolism of 2-nitrofluorene. Journal of Toxicology and Environmental Health, 47:587-599, 1996. **(E0672300) (KNOWLEDGE BASE) (Micro)**

186. Prapurna, R.D. and Rao, K.S. The long-term effects of caloric restriction initiated at different ages on DNA polymerases in rat brain. Mech. of Ageing & Dev. Accepted: 10/26/96. **(CONCEPT-DRIVEN) (Neuro Tox)**

187. Prapurna, R.D. and Rao, K.S. DNA polymerases delta and epsilon in developing and aging rat brain. International Journal of Developmental Neuroscience, Accepted: 3/01/96. **(CONCEPT-DRIVEN) (Neuro Tox)**

188. Pravdenkova, S.V., Basnakian, A.G., James-Gaylor, S.J., and Anderson, B.J. DNA fragmentation and nuclear endonuclease activity in rat brain after severe closed head injury. Brain Research, 729(2):151-155, 1996. **(CONCEPT-DRIVEN) (Bio Tox)**

189. Rafii, F. and Cerniglia, C.E. Role of reductive enzymes from human intestinal bacteria in the metabolism of azo dyes and nitro-polycyclic aromatic hydrocarbon. Microecol. Ther. 23:111-123, 1995. **(E0693200) (METHOD-DRIVEN) (Micro)**

190. Rafii, F., Moore, J.D., Ruseler-Van Embden, J.G.H. and Cerniglia, C.E. Bacterial reduction of azo dyes used in foods, drugs and cosmetics. Microecol. Ther. 25:147-156, 1995. **(E0693200) (METHOD-DRIVEN) (Micro)**

191. Rafii, F., Ruseler-Van Embden, J.H., Asad, Y.F., and Cerniglia, C.E. Azoreductase and nitroreductase activities of bacteria in feces from patients with an ileal reservoir. Digestive Diseases and Sciences, Accepted: 9/04/96 **(E0693201) (METHOD-DRIVEN) (Micro)**

192. Rajaratnam, V.S. Isolation, characterization and complete nucleotide sequence of a galleria mellonella cDNA coding for the follicle cell-specific yolk protein, YP4 Insect. Biochemistry and Molecular Biology, 26(6):545-555, 1996. **(CONCEPT-DRIVEN) (R&D Tox)**

193. Raza, H., Squires, R., Guengerich, F.P., Miller, D.W., and Freeman, J. P. Metabolism of 2-amino-a-carboline, a food-borne heterocyclic amine, mutagen and carcinogen, by human and rodent liver microsomes and by human cytochrome P-4501A2. Drug Metabolism, 24:395-400, 1996. **(E0694700) (Predictive Toxicity) (Mol Epi)**

194. Razzaghi, M. and Gaylor, D.W. On the correlation coefficient between the TD50 and the MTD. Risk Analysis, 16:107-113, 1996. **(S00116) (CONCEPT-DRIVEN) (Biometry)**

195. Reed, J.M. Communicating quality in a research environment. Arkansas Quality Conference Transactions, 1996. **(CONCEPT-DRIVEN) (Research)***

196. Ritter, C.L., Bennett, K.K., Fullerton, N.F., Beland, F.A., and Malejka-Giganti, D. Effects of ovariectomy on the *in vitro* and *in vivo* activation of carcinogenic N-2-fluorenyl hydroxamic acids by rat mammary gland and liver. Carcinogenesis, 17:2411-2418, 1996. **(E06970) (AGENT-DRIVEN) (Bio Tox)**

197. Rushing, L.G. and Thompson, H.C. Simultaneous determination of malachite green, gentian violet and their leuco metabolites in catfish or trout tissue by high performance liquid chromatography with visible detection. Journal of Chromatography B. Accepted: 6/27/96. **(METHOD-DRIVEN) (Chemistry)**

198. Sandberg, J.A., Duhart, H.M., Lipe, G.W., Binienda, Z.K., Slikker, W., Jr., and Kim, C. Disposition of 2,4-dichlorophenoxyacetic acid (2,4-D) in maternal and fetal rabbits. J. Toxicol. Environ. Health, Accepted: 3/18/96. **(E0681521) (PREDICTION OF TOXICITY) (Neuro Tox)**

199. Scallet, A.C., Ye, X., and Ali, S.F. *Nos* and *fos* in rat and mouse brain regions: possible relation to ibogaine-induced Purkinje cell loss. Annals New York Academy of Science, Accepted: 5/01/96. **(E0683000) (CONCEPT-DRIVEN) (Neuro Tox)**

200. Scallet, A.C., Ye, X., Rountree, R.L., Nony, P.A., and Ali, S.F. Ibogaine produces neurodegeneration in rat, but not mouse, cerebellum: neurohistological biomarkers of Purkinje cell loss. Annals New York Academy of Science, Accepted: 5/01/96. **(E0683000) (CONCEPT-DRIVEN) (Neuro Tox)**

201. Schmued, L.C., Albertson, C.M., Andrews, A., Nickols, J., and Sandberg, J.A. Evaluation of brain and nerve pathology in rats chronically dosed with ddl or isoniazid. Neurotoxicology and Teratology, Accepted: 3/13/96. **(E0672504) (PREDICTION OF TOXICITY) (Neuro Tox)**

202. Shaddock, J.G., Chou, M.W., and Casciano, D.A. Effects of age and caloric restriction on cell proliferation in hepatocyte cultures from control and hepatectomized Fischer 344 rats. Mutagenesis, 11(3):281-284, 1996. **(E0663600) (CONCEPT-DRIVEN) (Gen Tox)**

203. Sheldon, W.G., Bucci, T.J., Hart, R.W., and Turturro, A. Longevity and neoplasia in ad-libitum fed and food restricted $B_6D_2F_1$ mice fed NIH-31 open formula diet. Toxicologic Pathology, Accepted: 3/01/96. **(E0050100) (CONCEPT-DRIVEN) (Research)***

204. Shields, P.G., Ambrosone, C.B., Graham, S., Harrington, A.M., Gillenwater, K.A., Marshall, J.R., Vena, J.E., Laughlin, R., and Nemoto, T. A cytochrome P4502E1 genetic polymorphism (CYP2E1) and tobacco smoking in breast cancer. Molecular Carcinogeneis, 17:144-150, 1996. **(E0697800) (Predictive Toxicity) (Mol Epi)**

205. Shuttleworth, K.L. and Cerniglia, C.E. Practical methods for the isolation of polycyclic aromatic hydrocarbon (PAH)-degrading microorganisms and the determination of PAH mineralization and biodegradation intermediates. In: Manual of Environmental Microbiology, Chapter 84:766-775, 1996 (R.L. Crawford, ed., ASM Press) **(E0678700) (METHOD-DRIVEN) (Micro)**

206. Shuttleworth, K.L. and Cerniglia, C.E. Bacterial degradation of low concentrations of phenanthrene and inhibition by Naphthalene. Journal of Microbial Ecology, 31:305-317, 1996. **(E0678700) (PREDICTION OF TOXICITY) (Micro)**

207. Siitonen, P.H. and Ferguson, S.A. Determination of lead in maternal and weanling whole rat blood by flow injection ICP-MS. Proceedings of the 110th AOAC International Meeting in Orlando, Florida, 1996. **(METHOD-DRIVEN) (Chemistry)**

208. Silvers, K.J., Couch, L.H., Rorke, E.A., and Howard, P.C. The role of nitroreductases but not cytochrome P450 in the metabolic activation of 1-nitropyrene in the HepG2 human hepatoblastoma cell line. Biochem. Pharm., In Press, 1996. **(AGENT-DRIVEN) (Bio Tox)**

209. Slikker, W. and Gaylor, D.W. Letter to the Editor (FAAT). Fundamental and Applied Toxicology, Accepted: 5/06/96. **(PREDICTION OF TOXICITY) (Neuro Tox)**

210. Slikker, W., Jr. and Sobotka, T. Neurotoxicology-Risk Assessment: Food and Drug Administration Position. Proceedings of the 1996 Toxicology Forum, Accepted: 8/30/96. **(PREDICTION OF TOXICITY) (Neuro Tox)**

211. Slikker, W., Jr., Crump, K.S., Andersen, M.E., and Bellinger, D. Biologically- based, quantitative risk assessment of neurotoxicants. FAAT, 20:18-30, 1996. **(PREDICTION OF TOXICITY) (Neuro Tox)**

212. Slikker, W., Jr. Risk assessment for occupational neurotoxicants. Occupational Neurotoxicology, Accepted: 6/11/96. **(KNOWLEDGE-BASE) (Neuro Tox)**

213. Slikker, W. , Jr. The developing nervous system. Comprehensive Toxicology, Accepted: 5/01/96. **(PREDICTION OF TOXICITY) (Neuro Tox)**

214. Sobotka, T., Brown, R.L., Quander, D.Y., Jackson, R., Smith, M., Long, S., Barton, C.N., Hall, S., Hohannessen, J.N., and Scallet, A.C. Domoic acid: Neurobehavioral and neurohistological effects of low dose exposure in adult rats. Neurotoxicology and Teratology, Accepted: 4/12/96. **(E0681800) (AGENT-DRIVEN) (Neuro Tox)**

215. Sobotka, T., Ekelman, K.B., Slikker, W., Jr., Raffaele, R.K., and Hattan, D.G. Food and Drug Administration proposed guidelines for neurotoxicology testing of food chemicals. NeuroToxicology, 17(3):1-11, 1996. **(PREDICTION OF TOXICITY) (Neuro Tox)**

216. Sutherland, J.B., Evans, F.E., Freeman, J.P., and Williams, A.J. Biotransformation of quinoxaline by *Streptomyces badius*. Letters in Applied Microbiology, 22:199-201, 1996. **(E0678700) (AGENT-DRIVEN) (Micro)**

217. Syvertson, C.N. and Thompson, H.C. Evaluation and modification of a method for the determination of Dinoseb in potatoes and dried beans by HPLC using ion pairing and UV detection. Laboratory Infomation Bulletin DFS/ORO/ORA, 12:1LIB#4011, 1996 **(E0679700) (METHOD-DRIVEN) (Chemistry)**

218. Syvertson, C.N. and Thompson, H.C. GC-MS confirmation of bitertanol in bananas. Laboratory Information Bulletin DFS/ORO/ORA, 11#10:LIB3941, 1996. **(E0679700) (METHOD-DRIVEN) (Chemistry)**

219. Syvertson, C.N. and Thompson, H.C. GC-MS confirmation of dinoseb in potatoes and dried beans. Laboratory Information Bulletin, 4010:1-13, 1996. **(E0679800) (METHOD-DRIVEN) (Chemistry)**

220. Taurog, A., Dorris, M.L., and Doerge, D.R. Mechanism of simultaneous iodination and coupling catalyzed by thyroid peroxidase. Arch. Biochem. Biophys, 330:24-32, 1996. **(E0692001) (CONCEPT-DRIVEN) (Chemistry)**

221. Taurog, A., Dorris, M.L., and Doerge, D.R. Minocycline and the thyroid; antithroid effects of the drug, and the role of thyroid peroxidase in minocycline-induced black pigmentation of the gland. The Thyroid, 6(3):203-211, 1996. **(E0692001) (CONCEPT-DRIVEN) (Chemistry)**

222. Thompson, S.A., Harris, A.J., Hoang, D., Ferrer, M., and Johnson, G.R. C-terminal recombinant amphiregulin with bioactivity comparable to naturally-derived growth factor. Journal of Biological Chemistry, 271(30):17927-17931, 1996. **(CONCEPT-DRIVEN) (Gen Tox)**

223. Thurman, J.D., Greenman, D.L., Kodell, R.L., and Turturro, A. Oral squamous cell carcinoma in ad libitum fed and food-restricted Brown Norway. Toxicologic Pathology. Accepted: 8/06/96. **(E0050600) (CONCEPT-DRIVEN) (Biometry)**

224. Todotov, P., Cariuk, P., McDevitt, T., Coles, B., Fearon, K., and Tisdale, M. Characterization of a cancer cachectic factor. Nature, 379:739-742, 1996. **(CONCEPT-DRIVEN) (Bio Tox)**

225. Tolleson, W.H., Dooley, K.L., Sheldon, W.G., Thurman, J.D., Bucci, T.J., and Howard, P.C. The mycotoxin fumonisin induces apoptosis in cultured human cells and in livers and kidneys of rats. Fumonisins in Food, 21:237-250, 1996. **(E0211001) (AGENT-DRIVEN) (Bio Tox)**

226. Tolleson, W.H., Melchior, W.B., Morris, S.M., McGarrity, L.J., Domon, O.E., Muskhelishvili, L., James-Gaylor, S.J., and Howard, P.C. Apoptosis and antiproliferative effects of fumonisin B_1 in human keratinocytes, fibroblasts, esophageal epithelial cells, and hepatoma cells. Carcinogenesis, 17(2):239-249, 1996. **(E0211101) (AGENT-DRIVEN) (Bio Tox)**

227. Turturro, A., Duffy, P.H., Hart, R.W., and Allaben, W.T. Rationale for the use of dietary control in toxicity studies - $B_6C_3F_1$ mouse. Proceedings of the Toxicology Forum, In Press, 1996. **(CONCEPT-DRIVEN) (Cal Res)**

228. Unz, R.F. and Shuttleworth, K.L. Microbial mobilization and immobilization of heavy metals. Current Opinion in Biotechnology, 7(3):307-310, 1996. **(PREDICTION OF TOXICITY) (Micro)**

229. Valentine, C.R., Valentine, J.L., Seng, J.E., Leakey, J.E., and Casciano, D.A. The use of transgenic cell lines for evaluating toxic metabolites of carbamazepine. Cell Biology and Toxicology, 12:155-165, 1996. **(E0685600) (PREDICTION OF TOXICITY) (Gen Tox)**

230. Vanyushin, B.E. and Poirier, L.A. *Drosophila melanogaster* genomic DNA sequence homologous to mammalian cytosine DNA-methyltransferase gene. Biochemistry and Molecular Biology International, 39(2):353-358, 1996. **(E0687511) (CONCEPT-DRIVEN) (Mol Epi)**

231. Vanyushin, B.E., Lopatina, N.G., Wise, C.K., Fullerton, F.R., and Poirier, L.A. Butylated hydroxytoluene modulates DNA methylation in rats. European J. Biochemistry, In Press, 1996. **(E0667700) (CONCEPT-DRIVEN) (Mol Epi)**

232. Vollmer, D.L. and Lay, J.O. Characterization of nitrosation products in cosmetic-raw materials by various LC-MS techniques. Proceedings of the 44th ASMS Conference on Mass Spectrometry & Allied Topics, pg. 191, 1996. **(METHOD-DRIVEN) (Chemistry)**

233. Volmer, D. and Wilkes, J. G. Multiresidue analysis of sulfonylurea herbicides by on-line trace enrichment and liquid chromatography-mass spectrometry. Real-time Analytical Series, with Emphasis on Hyphenated Analytical Strategies, 1:259-267, 1996. **(METHOD-DRIVEN) (Chemistry)**

234. Volmer, D. and Wilkes, J.G. On-line postcolumn photolysis LC-Electrospray-MS analysis of isomeric triazine pesticides in food samples. Proceedings of the 44th ASMS Conference on Mass Spectrometry & Allied Topics, pg. 847, 1996. **(E0693101) (KNOWLEDGE BASE) (Chemistry)**

235. Volmer, D., Lay, J.O., Billedeau, S.M., and Vollmer, D.L. Detection and confirmation of N-nitrosodialkylamines using liquid chromatography-electrospray ionization coupled on-line with a photolysis reactor. Analytical Chemistry, 68(3):545-552, 1996. **(E0698901) (METHOD-DRIVEN) (Chemistry)**

236. Volmer, D., Lay, J.O., Billedeau, S.M., and Vollmer, D.L. Characterization of nitrosation products in cosmetic-raw materials by liquid chromatography-mass spectrometry techniques. Rapid Communications in Mass Spectrometry, 10:715-720, 1996. **(E0698901) (METHOD-DRIVEN) (Chemistry)**

237. Volmer, D., Wilkes, J.G., and Vollmer, D. Multiresidue analysis of pesticides by electrospray LC-MS and LC-MS/MS LC.GC. Magazine of Separation Science, 14 (3):216-224, 1996. **(E0693101) (KNOWLEDGE BASE) (Chemistry)**

238. Von Tungeln, L.S., Bucci, T., Hart, R.W., Kadlubar, F.F., and Fu, P.P. Inhibitory effect of caloric restriction on tumorigenicity induced by 4-aminobiphenyl and 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine (PhIP) in the CD1 newborn mouse bioassay. Cancer Letters, 104:133-136, 1996. **(E0657300) (PREDICTIVE TOXICITY) (Bio Tox)**

239. Walker, A.E., Pothuluri, J.V., Heinze, T.M., Volmer, D., and Cerniglia, C.E. Biosynthetic production of 13C and 14C erythronolide labeled erythromycin A. J. of Labelled Compounds and Radiopharmaceuticals, Accepted: 8/07/96. **(E0690101) (CONCEPT-DRIVEN) (Chemistry)**

240. Wang, R., Campbell, W.L., Cao, W., Summage-West, C.V., Steele, R.S., and Cerniglia, C. E. Detection of *Pasteurella pneumotropica* in laboratory mice and rats by PCR. Laboratory Animal Science, 46:81-85, 1996. **(E0687701) (METHOD-DRIVEN) (Micro)**

241. Wang, R., Cao, W., and Cerniglia, C.E. PCR detection and quantitation of predominant anaerobic bacteria in human and animal fecal samples. Applied and Environmental Microbiology, 62:1242-1247, 1996. **(E0687701) (METHOD-DRIVEN) (Micro)**

242. Wang, R., Cao, W., and Cerniglia, C.E. Phylogenetic analysis of *Fusobacterium prausnitzii* based upon 16S rRNA gene sequence and PCR confirmation. Int. J. Syst. Bacteriol., 46(1):341-343, 1996. **(E0687701) (METHOD-DRIVEN) (Micro)**

243. Wang, R., Henderson, A., Cao, W., and Cerniglia, C.E. PCR detection of polycyclic aromatic hydrocarbon-degrading Mycobacteria. Environmental Science & Technology, 30(1):307-311, 1996. **(E0687701) (METHOD-DRIVEN) (Micro)**

244. Wang, R.-F., Cao, W.-W., Johnson, M.G. and Cerniglia, C.E. Comparison of different PCR cycler machines for rapid and sensitive detection of pathogens. The Rapid Cyclist, 3:12-14, 1995. **(E0687701) (METHOD-DRIVEN) (Micro)**

245. Webster, P.A., Roberts, D.W., Benson, R.W., and Kearns, G.L. Chronic acetaminophen toxicity in children: diagnostic confirmation using a specific antigenic biomarker. J. Clin. Pharm., 36:397-402, 1996. **(E0677600) (PREDICTIVE TOXICITY) (Bio Tox)**

246. West, R.W., Hinson, W.G., and Swicord, M.L. Response for anchorage-independent growth with JB6 cells exposed to 60 hertz magnetic fields at several flux densities. Bioelectrochem. Bioenergetics, 39:175-179, 1996. **(E0677000) (AGENT-DRIVEN) (Bio Tox)**

247. Wilkes, J.G., Akbar, S. and Barry, T.L. Classifying analgesics by batch of origin using pyrolysis mass spectrometry with computerized pattern recognition. Proceedings of the 44th ASMS Conference on Mass Spectrometry, pg. 165, 1996. **(E0693101) (KNOWLEDGE-BASE) (Chemistry)**

248. Wilkes, J.G. and Vestal, M.L. Performance and theory for an HPLC to GC detector interface. Real-Time Analytics Series, with Emphasis on Hyphenated Analytical, 1:251-258,1996. **(P00321) (AGENT-DRIVEN) (Chemistry)**

249. Wilkes, J.G., Billedeau, S.M., Abramson, F., Volmer, D., and Teffera, Y. Parallel analyses using an HPLC interface with gas phase detectors. Real Time Analytics Series, with Emphasis on Hyphenated Analytical Strategies, 1:268-277, 1996. **(P00321) (AGENT-DRIVEN) (Chemistry)**

250. Wilkes, J.G., Churchwell, M.I., Billedeau, S.M., Vollmer, D.L.,Thompson, H.C., and Lay, J. O. Determination of underivatized fumonisin B_1 and related compounds by HPLC. ACS., Symposium "Fumonisin in Foods", Chpt. 8:93-103, 1996. **(AGENT-DRIVEN) (Chemistry)**

251. Wilkes, J.G., Sutherland, J.B., Rafii, F., Voorhees, K., Warden, C., Hall, R., Freeman, J.P., and Lay, J.O. Factors which affect the reproducibility of pyrolysis mass spectra for biological samples. Proceedings of the 44th ASMS Conference on Mass Spectrometry, pg. 589, 1996. **(E0693101) (KNOWLEDGE-BASE) (Chemistry)**

252. Wolff, G.L. and Hill, B.F. Accreditation of laboratory animal facilities: The Beginning Contemporary Topics in Laboratory Animal Science, 35(2):45-46, 1996. **(CONCEPT-DRIVEN) (Bio Tox)**

253. Wolff, G.L. Variability in gene expression and tumor formation within genetically homogeneous animal populations in bioassays. Fundamental and Applied Toxicology, 29:176-184, 1996. **(CONCEPT-DRIVEN) (Bio Tox)**

254. Ye, X. and Carp, R. I. Margination and diapedesis of inflammatory cells in the islets of Langerhans in hamsters infected with the 139H strain of scrapie. Journal of Comparative Pathology, 114:149-163, 1996. **(METHOD-DRIVEN) (Neuro Tox)**

255. Ye, X., Scallet, A.C., and Carp, R.I. The 139H scrapie agent produces hypothalamic neurotoxicity and pancreatic histopathology: Electron microscopic studies. NeuroToxicology, Accepted: 10/29/96. **(CONCEPT-DRIVEN) (Neuro Tox)**

256. Young, J.F. PBPK model for pregnancy as adjunct for investigation of developmental mechanisms. Proceedings for the 9th International Conference on Mechanics in Medicine and Biology, 9:295-298, 1996. **(PREDICTION OF TOXICITY) (R&D Tox)**

257. Young, J.F., Schwetz, B.A., Willhite, C.C., and Kacew, S. Symposium on Pharmacokinetics/ Pharmacodynamics in the Developing System and Impact on Risk Assessment: Executive Summary. J Toxicol Environ Hlth, 49:339-355, 1996. **(PREDICTION OF TOXICITY) (R&D Tox)**

258. Zhan, D., Heflich, R.H., and Fu, P.P. Molecular characterization of mutation and comparison of the mutation profile in the *hprt* gene of Chinese hamster ovary cells treated with benzo[a]pyrene trans-7,8-anti-9,10-epoxide, 1-nitrobenzo[a]pyrene trans-7,8-anti-9,10-epoxide, and 3-nitrobenzo[a]-pyrene trans-7,8-anti-9,10-epoxide. Environ. Mol. Mut., 27:19-29, 1996. **(E0657300) (PREDICTIVE TOXICITY) (Bio Tox)**

259. Zhang, D., Evans, F.E., Freeman, J.P., Yang, Y., Deck, J., and Cerniglia, C.E. Formation of mammalian metabolites of cyclobenzaprine by the Fungus, *Cunninghamella elegans*. Chemico-Biological Interactions, Accepted: 8/07/96 **(E0694301) (AGENT-DRIVEN) (Micro)**

260. Zhang, D., Freeman, J.P., Sutherland, J.B., Walker, A.E., Yang, Y., and Cerniglia, C.E. Biotransformation of chlorpromazine and methdilazine by *Cunninghamella elegans*. Appl. Environ. Microbiol., 62:798-803, 1996. **(E0694201) (PREDICTION OF TOXICITY) (Micro)**

261. Zhang, D., Hansen, E., Deck, J., Heinze, T.M., Henderson, A., Korfmacher, W.A., and Cerniglia, C.E. Fungal transformations of antihistamines: metabolism of cyproheptadine hydrochloride by *Cunninghamella elegans*. Xenobiotica, Accepted: 10/16/96. **(E0694201) (PREDICTION OF TOXICITY) (Micro)**

262. Zhang, D., Hansen, E., Deck, J., Heinze, T.M., Sutherland, J.B., and Cerniglia, C.E. Fungal biotransformation of the antihistamine azatadine by *Cunninghamella elegans*. Applied and Environmental Microbiology, 62(9):3477-3479, 1996. **(E0694201) (PREDICTION OF TOXICITY) (Micro)**

263. Zhang, D., Yang, Y., Leakey, J.E., and Cerniglia, C.E. Phase I and Phase II enzymes produced by *Cunninghamella elegans* for the metabolism of xenobiotics. FEMS Microbiology Letters, 138:221-226, 1996. **(METHOD-DRIVEN) (Micro)**

264. Zwicker, G.M. and Eyster, R.C. Proliferative bone lesions in rats fed a diet containing glucocorticoid for up to two years. Toxicologic Pathology, 24(2):246-250, 1996. **(CONCEPT-DRIVEN) (Research)***

265. Zwicker, G.M., Gosselin, S.J., Solomon, J.L., and Hurst, G.H. Effects found in a one-year oral toxicity study in Sprague-Dawley Rats of a Novel 5-HT1a receptor partial agonist for the treatment of anxiety in humans. Toxicologic Pathology, 24:207-213, 1996. **(AGENT-DRIVEN) (Research)***

***Research Publications are publications in support of NCTR research which are not directly linked to a specific research area.**

INDICES

CHEMCIAL INDEX

Chemical Index